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(57) Abstract

Therapeutic compounds, having at least one aldehyde or ketone-substituted side chain have the formula: CORE MOIETY — $(R)_j$, including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof. j is an integer from one to three; the core moiety is non-cyclic or cyclic; and R may be selected from the group consisting of hydrogen, halogen, hydroxy, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups, and formula (I): — $(CH_2)_n$ — C — $(R_1)_p$. At least one R has formula (I) and n is an integer from three to twenty; p is two or three; and R_1 is selected from the group consisting of hydrogen, halogen, hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group, =O, $-(CH_2)_s-C(R_2)_t$, (wherein s is zero or an integer form one to ten, t is two or three, R_2 is hydrogen, halogen, hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ ether, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group, or =O). At least one R_1 or one R_2 is =O; and a second R_1 or second R_2, bonded to the same, respective -C as the at least one R_1 or one R_2, is other than =O or hydroxyl. The compounds and pharmaceutical compositions thereof are useful as therapies for diseases advanced via intracellular signaling through specific intracellular signaling pathways by mediating a signaling response to an external stimuli.

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CELL SIGNALING INHIBITORS

Technical Field of the Invention

The invention provides a group of compounds that are effective agents to inhibit specific cellular signaling events often induced by inflammatory stimuli, or to be directly or indirectly antimicrobial to yeast or fungal infections. More specifically, the inventive compounds have at least one aldehyde or ketone-substituted chain bonded to a core moiety. The inventive compounds are useful in treating or preventing a variety of diseases, wherein the disease is characterized by or can be treated by inhibiting an immune response or a cellular response to external or *in situ* primary stimuli.

Background of the Invention

Pentoxifylline (1-(5-oxohexyl)-3,7-dimethylxanthine), abbreviated PTX, is a xanthine derivative which has seen widespread medical use for the increase of blood flow. PTX is disclosed in U.S. Patents Nos. 3,422,107 and 3,737,433, both to Mohler et al. Metabolites of PTX were summarized in Davis et al., *Applied Environment Microbiol.* 48:327, 1984. A metabolite of PTX is 1-(5-hydroxyhexyl)-3,7-dimethylxanthine, designated M1. M1 was also disclosed as increasing cerebral blood flow in U.S. Patents Nos. 4,515,795 and 4,576,947 to Hinze et al. Another metabolite, 1-(5-oxohexyl)-3-methylxanthine, designated M6, was disclosed by Bryce et al., *Arzneim.-Forsch./Drug Res.* 39(4):512-517, 1989. In addition, U.S. Patents Nos. 4,833,146 and 5,039,666 to Gebert et al. and Novick, Jr., respectively, disclose use of tertiary alcohol analogs of xanthine for enhancing cerebral blood flow.

PTX and its known metabolites thereof have been shown to have *in vivo* activity in specific biologic systems. U.S. Patent No. 4,636,507 to Kreutzer et al. describes an ability of PTX and M1 to further promote chemotaxis in polymorphonuclear leukocytes responding to a chemotaxis stimulator. In addtion, PTX and related tertiary alcohol substituted xanthines inhibit activity of certain cytokines to affect chemotaxis (U.S. Patents Nos. 4,965,271 and 5,096,906 to Mandell et al.). By administrating PTX and GM-CSF, patients undergoing allogeneic bone marrow transplant exhibited decreased levels of tumor necrosis factor, TNF, (Bianco et al., *Blood* 76: Supplement 1 (522A), 1990). Reduction in assayable levels of TNF was accompanied by a reduction in bone marrow transplant-related complications. However, in normal volunteers, TNF levels were higher among PTX recipients. Therefore, elevated levels of TNF are not the primary cause of such complications.

Further research with PTX, its metabolites and their activity relating to various biologic systems spurred investigations with potential therapeutic agents heretofore unknown.

These agents were identified as potential therapies for treating or preventing disease by inhibiting secondary cellular response to an external or *in situ* primary stimuli. These investigations sought to identify efficacious therapeutic compounds which were safe and effective for human or animal administration and maintain cellular homeostasis in the face of a variety of inflammatory stimuli.

Compounds disclosed herein and discovered in search of potential disease treatments which would prevent or treat a disease with minimal or no adverse side effects, have biologic activity in various, predictive assays. The inventive compounds exhibit utility in preventing an undesireable cellular response to noxious stimuli. Results from predicitive assays indicate that these inventive compounds have potential as therapies in treating a broad spectrum of clinical indications, acting via a variety of disease mechanisms. However, all these mechanisms appear to affect the second messenger pathway. Results of this research are the subject matter of this disclosure, the compounds discussed herein having novel structures and remarkable and surprising properties heretofore unknown.

Summary of the Invention

The invention is directed to aldehyde- or ketone-substituted therapeutic compounds, pharmaceutical compositions and uses thereof. The inventive aldehyde- or ketone-substituted compounds are useful in a large variety of therapeutic indications for treating or preventing disease. In particular, the inventive compounds and pharmaceutical compositions thereof provide therapy for diseases characterized or advanced by a variety of cellular responses to primary stimuli, the cellular response effected by secondary signaling through specific intracellular signaling pathways, more specifically the pathways herein discussed. Treatment may mediate a signaling response to an external stimuli. This intracellular signaling is characteristic of diseases treatable using the inventive compounds or pharmaceutical compositions thereof.

The inventive compounds have at least one aldehyde- or ketone-containing side chain and are preferably cyclic or heterocyclic compounds. The inventive compounds and pharmaceutical compositions thereof have the formula:

$$\text{CORE MOIETY} \text{ —— } (R)_j$$

including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof, wherein j is an integer from one to three, the core moiety is non-cyclic or cyclic and R may be selected from among: hydrogen, halogen (preferably bromine, chlorine, fluorine and

iodine), hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups, and formula I.

The inventive compounds have at least one R of the following formula I:

$$-(CH_2)_n-C-(R_1)_p \quad \text{I}$$

wherein n is an integer from three to twenty; p is two or three; R_1 is selected from among hydrogen, halogen, hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group, =O, $-(CH_2)_S-C(R_2)_t$ (wherein s is zero or an integer from one to ten, t is two or three, R_2 is hydrogen, halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group, or =O); at least one R_1 or one R_2 is =O; and a second R_1 or second R_2, bonded to the same, respective -C as the at least one R_1 or one R_2, is other than =O or hydroxyl.

The invention also provides a pharmaceutical composition. Pharmaceutical compositions of the inventive compounds comprise a pharmaceutical carrier, diluent or excipient and some amount of an inventive compound.

The invention provides a method for treating an individual having a variety of diseases. The disease is characterized by or can be treated by inhibiting an immune response or a cellular response to external or *in situ* primary stimuli. Indications useful for administering compounds of the invention include, but are not limited to: the presence of a tumor burden, a hormone-related disorder, a neurological disorder, an autoimmune disease, inflammation, restenosis, coronary artery disease, atherosclerosis, hypertension, unwanted immune response (such as allograft reactions), viral infection, nephritis, mucositis, and various allergic responses. Allergic responses include acute allergic response and thus rhinorrhea, sinus drainage, diffuse tissue edema, and generalized pruritus. As well as the following, other chronic allergic responses include, dizziness, diarrhea, tissue hyperemia, and lacrimal swelling with localized lymphocyte infiltration. Allergic reactions are also associated with leukotriene release and the distal effects thereof, including asthmatic symptoms (*e.g.*, development of airway obstruction, a decrease in FEV1, changes in vital capacity, and extensive mucus production).

Brief Description of the Drawings

Figure 1 is a dose response curve prepared from results in a murine thymocyte assay determining inhibitive effects of inventive compound no. 1595 (see below for chemical name and structure) on thymocyte proliferation.

Figure 2 is a dose response curve in the murine thymocyte assay for inventive compound no. 2510.

Figures 3, 4, 5 and 6 are plotted graphs of compound concentrations (μM) for compounds nos. 1516, 1526, 1810 and 2594, respectively, versus incorporated thymidine (cpm) in an *in vitro* mixed lymphocyte (MLR) assay useful in determining biologic activity of the compounds.

Figure 7 compares results obtained in an assay comparing an effect on normal cells of inventive compounds identified as potential cancer therapies to a known chemotherapeutic agent.

Figure 8 represents experimentally determined effects of inventive compound no. 2591 on percent adherence of Jurkat cells to TNFα-stimulated human umbilical vein endothelial cells (HUVEC).

Figure 9 corresponds to data obtained for inventive compound no. 2591 on adherence of THP-1 cells to TNFα-stimulated HUVEC.

Figure 10 illustrates an ability of compound no. 2594 to inhibit adherence of THP-1 cells to TNFα-stimulated HUVEC.

Figures 11 and 12 show an ability of inventive compound no. 2591 to inhibit THP-1 and neutrophil cell adherence to IL-1β stimulated HUVEC, respectively.

Figure 13, 14, 15 and 16 report % surface expression of intracelluar adhesion molecule (ICAM) or vascular cell adhesion molecule (VCAM) in HUVEC--as a function of mean fluorescence intensity--versus concentration of inventive compound.

Figure 17 reports data obtained in a Balb/3T3 cell proliferation assay, illustrating inhibitive effects of the inventive compounds on proliferation in this system.

Figure 18 illustrates that the inventive an inventive compound tested in the Balb/3T3 cell proliferation assay of figure 16 is not cytotoxic to cells used in this assay.

Figure 19 reports inhibitive results obtained for several inventive compounds in a lipo-protein saccharide (LPS)-induced TNF release assay using whole human blood.

Figure 20a reports results obtained in a murine thymocyte proliferation assay for comparative compounds 1-(5-oxohexyl)-3-methylxanthine (M6) and 1-(5-oxohexyl)-3,7-dimethylxanthine.

Figure 20b illustrates results for inventive compound no. 1516 in a murine thymocyte assay used in comparing activity of the inventive compounds with comparative compounds represented in figure 20a.

Detailed Description of the Invention

The invention provides a genus of compounds which can control cellular behavior by a particular phase of a secondary messenger pathway system (Bursten et al., *J. Biol. Chem.* 266:20732, 1991). The second messengers are lipids or phospholipids and use the following abbreviations:

PE = phosphatidyl ethanolamine
LPE = lysophosphoethanolamine
PA = phosphatidic acid
LPA = lysophosphatidic acid
DAG = diacylglycerol
LPLD = lysophospholipase-D
LPAAT = lysophosphatidic acid acyl transferase
PAPH = phosphatidic acid phosphohydrolase
PLA2 = phospholipase A2.
PLD = phospholipase D
PAA = phosphoarachidonic acid
PC = phosphatidyl choline

"remodeled" PA, cyclic pathway = PAA, LPA, PA and DAG intermediates substituted with l-saturated, 2-linoleoyl or 1,2-dioleoyl, dioleoyl/1,2-sn-dilinoleoyl at the indicated sn-1 and sn-2 positions.

"Classical PI Pathway" = PI, DAG, PA intermediates substituted with 1-stearoyl, 2-arachidonoyl fatty acyl side chains.

"PLD-generated PA" = PE, PC, LPA, PA and DAG intermediates substituted with, *e.g.*, 1,2-sn-dioleoyl-, 1-alkyl, 2-linoleoyl-, and 1-alkyl, 2-docosahexaenoyl-side chains.

Lysophosphatidic acid transferase (LPAAT) effects the synthesis of phosphatidic acid (PA) from lysophosphatidic acid (LPA) by incorporation of an acyl group from acyl CoA. Hydrolysis of the phosphate moiety by PA phosphohydrolase (PAPH) results in the formation of DAG. These aspects of the pathway appear to be activated immediately (within a minute) upon stimulation by a primary stimulus (*e.g.*, a cytokine such as IL-1, IL-2 or TNF) acting at a receptor on a cellular surface. An immediate detectable effect is an elevation of levels of PA and DAG. Administration of the compounds of the invention reverse this elevation.

The compounds and pharmaceutical compositions of the invention include inhibitors of subspecies of LPAAT and PAPH enzymes with substrate specificity for intermediates with 1,2-diunsaturated and 1-alkyl, 2-unsaturated subspecies. One representative example of such an inhibitor (although not within the genus of inventive compounds) is PTX. PTX blocks PAPH in a specific activation pathway that does not involve PI but rather derives

from a PA that is largely composed of 1,2-diunsaturated and 1-alkyl, 2-unsaturated subspecies. This was shown, for example, by the demonstration that human mesangial cells stimulated with TNF produce DAG from PI and regenerate PI in the absence and the presence of PTX. In the latter system there is no evidence to suggest that PA or DAG are derived from sources other than PI. It should be emphasized that the compounds of the invention affect that subset of PAPH and LPAAT that relates to substrates with unsaturated fatty acids other than arachidonate in the sn-2 position, not the housekeeping forms of these enzymes that serve the PI pathway.

Each membrane phospholipid subclass (*e.g.*, PA, PI, PE, PC and PS) reaches a stable content of characteristic fatty acyl side chains due to cyclic remodeling of the plasma membrane as well as turnover for each subclass. PA is often stable, but present in relatively small quantities. PA in resting cells consists mostly of saturated acyl chains, usually consisting of myristate, stearate and palmitate. In resting cells, PC's acyl side chains consist mostly of acyl palmitate in the sn-1 position and oleate in the sn-2 position. PE and PI are predominantly composed of sn-1 stearate and sn-2 arachidonate.

Due to this characteristic content of acyl groups in the sn-1 and sn-2 positions, the origin of any PA species may be deduced from the chemical nature of its acyl groups in the sn-1 and sn-2 positions. For example, if PA is derived from PC through action of the enzyme PLD, the PA will contain the characteristic acyl side chains of PC substrate passed through the second messenger pathway. Further, the origin of any 1,2 sn-substrate species may be differentiated as to its origin. It is important to know whether or not each phospholipid species passes through a PA form prior to hydrolysis to DAG. The lyso-PA that is converted to PA and then to DAG may be shown. The complexities of this second messenger pathway can be sorted by suitable analyses using fatty acyl side chain chemistry (*e.g.*, by thin layer chromatography, gas-liquid chromatography, or high pressure liquid chromatography) of intermediates in cells at various time points after stimulation of the second messenger pathway.

In certain mesenchymal cells, such as neutrophils and rat or human mesangial cells, several signaling pathways may be activated in tandem, simultaneously or both. For example, in neutrophils, F-Met-Leu-Phe stimulates formation of PA through the action of PLD, followed in time by formation of DAG through PAPH action. Several minutes later, DAG is generated from PI through the classical phosphoinositide pathway. In many cells, DAG is derived from both PA that is remodeled through a cycle whereby PA is sn-2 hydrolyzed by PLA2, followed by sn-2 transacylation by LPAAT and PA that is generated in a PLD-pathway from either PE or PC or both substrates by PLD.

The present second messenger pathway involves substrates with unsaturated fatty acids in the sn-2 position other than arachidonate and those sub-species of PAPH and LPAAT that are not involved in normal cellular housekeeping functions that are part of the classical PI

pathway. The PAPH and LPAAT enzymes involved in this specific second messenger pathway are exquisitely stereo-specific for different acyl side chains and isomeric forms of substrates. Therefore, the inventive compounds may preferably be substantially enantiomerically pure.

PTX (*in vitro*) blocks formation of remodeled PA through the PA/DAG pathway at high PTX concentrations (greater than those that could be achieved in patients without dose-limiting side effects) by blocking formation of PA subspecies at LPAAT. Even in the presence of PTX, cells continue to form PA through the action of PLD, and DAG is also formed through the action of phospholipase C on PC and PI. The latter pathway are not inhibited by the inventive compounds or PTX. In PTX-treated cells, DAG derived from remodeled and PLA-generated PA is diminished (*e.g.*, 1,2-sn-dioleoyl DAG, 1-alkyl, 2-linoleoyl DAG and 1-alkyl, 2-docosahexaneolyl DAG). Therefore, the inventive compounds inhibit the formation of only a certain species of PA and DAG by selectively inhibiting a specific second messenger pathway that is only activated in cells by noxious stimuli, but is not used to signal normal cellular housekeeping functions.

Therapeutic Uses of the Inventive Compounds

The specific inhibition of the second messenger pathway, as described above and activated primarily by various noxious stimuli, suggests that the inventive compounds are useful in treating a wide variety of clinical indications, mediated at the cellular level by a common mechanism of action. Moreover, *in vitro* and *in vivo* data presented herein provides predictive data that a wide variety of clinical indications, having similar effects on the specific second messenger pathway (activated by noxious stimuli and mediated through, for example, inflammatory cytokines), may be treated by the inventive compounds, which specifically inhibit the pathway. In fact, the mechanism of action for the inventive compounds explains why these compounds have multifarious clinical indications.

Activation of the second messenger pathway is a major mediator of response to noxious stimuli and results in cellular signals that lead to, for example, acute and chronic inflammation, immune response and cancer cell growth. Although the inventive compounds may desirably inhibit other noxious stimuli not discussed, they most effectively mediate the above conditions. Signals mediated by the present second messenger pathway include, for example, those cellular responses of LPS directly; T cell activation by antigen; B cell activation by antigen, cellular responses to IL-1, mediated through the IL-1 Type I receptor (but not the IL-1 Type II receptor), and TNF (Type I receptor), growth stimulated by transformations including, but not limited to, activated oncogenes (*e.g.*, *ras*, *abl*, *her 2-neu* and the like), smooth muscle cell proliferation stimulated by PDGF, b-FGF and IL-1; T cell and B cell growth stimulation by IL-2, IL-4 or IL-7 and IL-4 or IL-6, respectively; and more generally, T cell receptor signaling.

In vitro, the inventive compounds: (1) block IL-1 signal transduction through the Type 1 receptor as shown, for example, by preventing IL-1 and IL-1 plus PDGF (platelet derived growth factor) induction of proliferation of smooth muscle, endothelial and kidney mesengial cells; (2) suppress up-regulation of adhesion molecules as shown, for example, by blocking VCAM in endothelial cells; (3) inhibit TNF, LPS and IL-1 induced metalloproteases (an inflammation model); (4) block LPS, TNF or IL-1 induced metalloprotease and secondary cytokine production (for prevention and treatment of septic shock); (5) suppress T cell and B cell activation by antigen, for example, IL-2 and IL-4; (6) inhibit mast cell activation by IgE; (7) are cytotoxic for transformed cells and tumor cell lines, yet not for normal cells; and (8) block signaling by IL-2, IL-4, IL-6 and IL-7 on T and B cells.

The foregoing *in vitro* effects give rise to the following *in vivo* biological effects, including, but not limited to: protection and treatment of endotoxic shock and sepsis induced by gram positive or gram negative bacteria; inhibition of tumor cell growth; synergistic immunosuppression, active in autoimmune diseases and in suppressing allograft reactions; and stimulation of hair grow through reversal of an apoptotic process. The inventive compounds are most potent when used to prevent and treat septic shock, treat acute and chronic inflammatory disease, treat or prevent an autoimmune disease and stimulate hair growth (when applied topically).

The inventive compounds also are useful as an adjuvant to inhibit toxic side effects of drugs whose side effects are mediated through the present second messenger pathway.

Metalloproteases mediate tissue damage such as glomerular diseases of the kidney, joint destruction in arthritis, and lung destruction in emphysema, and play a role in tumor metastases. Three examples of metalloproteases include a 92 kD type V gelatinase induced by TNF, IL-1 and PDGF plus bFGF, a 72 kD type IV collagenase that is usually constitutive and induced by TNF or IL-1, and a stromelysin/PUMP-1 induced by TNF and IL-1. The inventive compounds can inhibit TNF or IL-1 induction of the 92 kD type V gelatinase inducable metalloprotease. Moreover, the inventive compounds can reduce PUMP-1 activity induced by 100 U/ml of IL-1. Accordingly, the inventive compounds prevent induction of certain metalloproteases induced by IL-1 or TNF and are not involved with constitutively produced proteases (*e.g.*, 72 kD type IV collagenase) involved in normal tissue remodeling.

The inventive compounds inhibit signal transduction mediated through the Type I IL-1 receptor, and are therefore considered as IL-1 antagonists. A recent review article entitled "The Role of Interleukin-1 in Disease" (Dinarello et al., *N. Engl. J. Med.* 328, 106, Jan. 14, 1993) described the role of IL-1 as "an important rapid and direct determinant of disease... In septic shock, for example, IL-1 acts directly on the blood vessels to induce vasodilatation through the rapid production of platelet activating factor and nitric oxide, whereas in

autoimmune disease it acts by stimulating other cells to produce cytokines or enzymes that then act on the target tissue." The article describes a group of diseases that are mediated by IL-1, including sepsis syndrome, rheumatoid arthritis, inflammatory bowel disease, acute and myelogenous leukemia, insulin-dependent diabetes melli... atherosclerosis and other diseases including transplant rejection, graft versus host disease (GVHD), psoriasis, asthma, osteoporosis, periodontal disease, autoimmune thyroiditis, alcoholic hepatitis, premature labor secondary to uterine infection and even sleep disorders. Since the inventive compounds inhibit cellular signaling through the IL-1 Type I receptor and are IL-1 antagonists, the inventive compounds are useful for treating all of the above-mentioned diseases.

For example, for sepsis syndrome, the mechanism of IL-1-induced shock appears to be the ability of IL-1 to increase the plasma concentrations of small mediator molecules such as platelet activating factor, prostaglandin and nitric oxide. These substances are potent vasodilators and induce shock in laboratory animals. Blocking the action of IL-1 prevents the synthesis and release of these mediators. In animals, a single intravenous injection of IL-1 decreases mean arterial pressure, lowers systemic vascular resistance, and induces leukopenia and thrombocytopenia. In humans, the intravenous administration of IL-1 also rapidly decreases blood pressure and doses of 300 ng or more per kilogram of body weight may cause severe hypotension. The therapeutic advantage of blocking the action of IL-1 resides in preventing its deleterious biological effects without interfering with the production of molecules that have a role in homeostasis. The present inventive compounds address this need, identified by Dinarello et al., by inhibiting cellular signaling only through the IL-1 Type I receptor and not through the IL-1 Type II receptor.

With regard to rheumatoid arthritis, Dinarello and Wolff state: "Interleukin-1 is present in synovial lining and synovial fluid of patients with rheumatoid arthritis, and explants of synovial tissue from such patients produce IL-1 *in vitro*. Intraarticular injections of interleukin-1 induce leukocyte infiltration, cartilage breakdown, and periarticular bone remodeling in animals. In isolated cartilage and bone cells *in vitro*, interleukin-1 triggers the expression of genes for collagenases as well as phospholipases and cyclooxygenase, and blocking its action reduces bacterial-cell-wall-induced arthritis in rats." Therefore, the inventive compounds, as IL-1 antagonists, are useful to treat and prevent rheumatoid arthritis.

With regard to inflammatory bowel disease, ulcerative colitis and Crohn's disease are characterized by infiltrative lesions of the bowel that contain activated neutrophils and macrophages. IL-1 can stimulate production of inflammatory eicosanoids such as prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) and IL-8, an inflammatory cytokine with neutrophil-chemoattractant and neutrophil-stimulating properties. Tissue concentrations of PGE2 and LTB4 correlate to severity of disease in patients with ulcerative colitis, patients with

inflammatory bowel disease having high tissue concentrations of IL-1 and IL-8. Therefore, an IL-1 antagonist, such as the inventive compounds, would be effective to treat inflammatory bowel disease.

With regard to acute and chronic myelogenous leukemia, there is increasing evidence that IL-1 acts as a growth factor for such tumor cells. Therefore, the inventive compounds should be effective to prevent the growth of worsening of disease for acute and chronic myelogenous leukemias.

Insulin-dependent diabetes mellitus (IDDM) is considered to be an autoimmune disease with destruction of beta cells in the islets of Langerhans, mediated by immunocompetent cells. Islets of animals with spontaneously occurring IDDM (*e.g.*, BB rats or NOD mice) have inflammatory cells that contain IL-1. Therefore, the inventive compounds should be useful for the preventing and treating IDDM.

IL-1 also plays a role in atherosclerosis development. Endothelial cells are a target of IL-1. IL-1 stimulates proliferation of vascular smooth muscle cells. Foam cells, isolated from fatty arterial plaques from hypercholesterolemic rabbits, contain IL-1β and IL-1β messenger RNA. The uptake of peripheral blood monocytes results in initiation of IL-1 production by these cells. IL-1 also stimulates production of PDGF. Taken together, IL-1 plays a part in the development of atherosclerotic lesions. Therefore, an IL-1 antagonist, such as the inventive compounds should be useful in preventing and treating atherosclerosis.

IL-1 activates (through the Type I IL-1 receptor) a lyso-PA acyltransferase (LPAAT) and phosphatidate phosphohydrolase within 5 seconds of cell (for example, human mesangial cells, HMC) exposure to this cytokine. As discussed in detail above, activation of both enzymes results in production of PA species with sn-1 and sn-2 unsaturated acyl groups, with the majority of sn-2 acyl chains being polyunsaturated. Both IL-1 and a product of LPAAT, 1,2-sn-dilinoleoyl PA, activate a signaling pathway involving hydrolysis of PE to PA. This reaction is followed by dephosphorylation of PA to produce both 1,2-sn-diacylglycerol, and 1-o-alkyl, or 1-o-alkenyl,acylglycerol (AAG) species. The inventive compounds exert their activity by inhibiting one or both enzymes at an inner leaflet of the plasma membrane. Therefore, appropriate *in vitro* models for drug activity may measure inhibition of stimulation caused by a proinflammatory cytokine or other inflammatory cellular signal.

The generation of the sn-2 unsaturated PA fraction by LPAAT serves to activate either G-proteins, or acts directly upon PLD through alteration of its lipid microenvironment. Activation of LPAAT and generation of the sn-2-unsaturated PA species is an energy sensitive pathway of PLD. This provides a mechanism for a limited-receptor system to amplify a signal and generate a cellular response by rapid synthesis of small amounts of PA. Uptake of di-unsaturated PA, which is less than about 0.1% of total membrane lipid mass, is sufficient to

activate PLD activity. This quantity of PA is similar to that endogeneously synthesized by LPAAT. The PA-stimulated PLD acts upon PE, which should be localized to the inner leaflet of the cell membrane, enriched in PE relative to the outer leaflet. Therefore, the cellular inflammatory response to IL-1 is mediated by the pathway: IL-1R → PA → (PLD) → PE. Whereas a localized tissue response is: lysoPA → PI→ PKC → (PLD) → PC. The PLD species are likely to be different isozymes. The second messenger pathway whose activation is inhibited by the inventive compounds is not a PI-derived pathway and does not involve PKC in the time courses of inhibition. PKC is acutely activated by PI-derived DAG, but chronic activation (*i.e.*, > 30 minutes) is maintained by PC-derived PA generated by PC-directed PLD. Therefore, the pathway inhibited by the inventive compounds is PE-directed and not PC-directed. Moreover, the PE-directed PLD favors substrates with sn-2 long-chain unsaturation.

DAG and PA are upregulated in oncogenically transformed cells. For example, activating *ras* mutations result in increased generation of DAG upon stimulation with mitogens, although the sources of DAG differ between experimental systems. In nontransformed renal mesangial cells, IL-1β stimulation increased PLA2 and LPAAT activation, resulting in generation of sn-2 unsaturated PA and subsequent hydrolysis to DAG by phosphatidate phosphohydrolase. The *ras* transformation in NIH/3T3 cells upregulates serum-stimulated generation of DAG and PA. Particular species of DAG that is stimulated by serum is dioleoyl and of PA are dilinoleoyl and dioleoyl. This upregulation occurs over 4-12 hours and pretreatment of cells with an inventive compound, or PTX, blocks generation of these phospholipid second messengers. The inhibition occurs either through suppressing the generation of PA *de novo* from lysoPA, or through inhibition of one or both arms of the Lands cycle. The coordinate increase of lysoPA in the setting of diminished PA/DAG production suggests inhibition of transacylation of a precursor lipid. Therefore, the *ras* transformation mediates an upregulation of PA through indirect stimulation of PLA2 and/or LPAAT activity. The inventive compounds inhibit the conversion of the upregulated lysoPA to PA and subsequently block the phenotypic changes induced by PA/DAG in the membrane.

The ability of the inventive compounds to inhibit generation of unsaturated phospholipids is mirrored by the ability of inventive compounds to inhibit proliferation and tumorogenicity of *ras*-transformed cells *in vitro* and *in vivo*. PTX inhibits *ras*-transformed NIH/3T3 cells more than parental cells. This inhibition is reversible and is not associated with significant cytotoxicity.

Excessive or unregulated TNF (tumor necrosis factor) production is implicated in mediating or exacerbating a number of diseases including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress

syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft versus host reaction, allograft rejections, fever, myalgias due to infection such as influenza, cachexia secondary to infection, AIDS or malignancy, AIDS, other viral infections (*e.g.*, CMV, influenza, adenovirus, herpes family), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, or pyresis. The inventive compounds or pharmaceutically acceptable salts thereof can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of any disease state in a human or other mammal, which is exacerbated or signaled through the present second messenger cellular phospholipid-based signaling pathway and by excessive or unregulated production of "first messenger" inflammatory cytokines such as TNF or IL-1. With regard to TNF first messenger signaling, there are several disease states in which excessive or unregulated TNF production by monocytes/macrophages is implicated in exacerbating or causing the disease. These include, for example, neurodegenerative diseases such as Alzheimers disease, endotoxemia or toxic shock syndrome (Tracey et al., *Nature* 330:662, 1987 and Hinshaw et al., *Circ. Shock* 30:279, 1990); cachexia (Dezube et al., *Lancet* 355:662, 1990), and adult respiratory distress syndrome (Miller et al., *Lancet* 2(8665):712, 1989). The inventive compounds may be used topically in the treatment of prophylaxis of topical disease states mediated or exacerbated by excessive TNF or IL-1, such as viral infections (herpes or viral conjunctivitis), psoriasis, fungal or yeast infections (ringworm, athletes foot, vaginitis, dandruff, etc.) or other dermatologic hyperproliferative disorders. High TNF levels have been implicated in acute malaria attacks (Grau et al., *N. Engl. J. Med.* 320:1585, 1989), chronic pulmonary inflammatory diseases such as silicosis and asbestosis (Piguet et al., *Nature* 344:245, 1990, and Bissonnette et al., *Inflammation* 13:329, 1989), and reperfusion injury (Vedder et al., *Proc. Natl. Acad. Sci. USA* 87:2643, 1990).

The compounds of the invention can inhibit certain VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor) and PDGF (platelet derived growth factor) effects *in vivo*, such as inhibition of angiogenesis or restenosis. For example, Ferns et al., *Science* 253:1129, 1991, have shown that neointimal smooth muscle chemotaxis and angioplasty are inhibited in rats using a neutralizing antibody to PDGF. Also, Jawien et al., *J. Clin Invest.* 89:507, 1992, have shown that PDGF promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. Inhibition of the PDGF-mediated effects following balloon angioplasty by the inventive compounds is the pharmacological rationale for using the inventive compounds as therapeutic agents to prevent restenosis. The inventive compounds also inhibit atherogenesis because increased levels of PDGF expressed by macrophages are associated with all phases of atherogenesis (Ross et al., *Science* 248:1009, 1990). Further, many human tumors express elevated levels of either PDGF, FGF, receptors for FGF or PDGF, or mutated

cellular oncogenes highly homologous to these growth factors or their receptors. For example, such tumor cell lines include sarcoma cell lines (Leveen et al., *Int. J. Cancer* 46:1066, 1990), metastatic melanoma cells (Yamanishi et al., *Cancer Res.* 52:5024, 1992), and glial tumors (Fleming et al., *Cancer Res.* 52:4550, 1992).

The inventive compounds are also useful to raise the seizure threshold, to stabilize synapses against neurotoxins such as strychnine, to potentiate the effect of anti-Parkinson drugs such as L-dopa, to potentiate the effects of soporific compounds, to relieve motion disorders resulting from administration of tranquilizers, and to diminish or prevent neuron overfiring associated with progressive neural death following cerebral vascular events such as stroke. In addition, the compounds of the invention are useful in the treatment of norepinephrine-deficient depression and depressions associated with the release of endogenous glucocorticoids, to prevent toxicity to the central nervous system of dexamethasone or methylprednisolone, and to treat chronic pain without addiction to the drug. Further, the compounds of the invention are useful in the treatment of children with learning and attention deficits and generally improve memory in subjects with organic deficits, including Alzheimer's patients.

Compounds of the Invention

The inventive compounds have at least one aldehyde- or ketone-containing side chain and are preferably cyclic or heterocyclic compounds. The inventive compounds and pharmaceutical compositions thereof have the formula:

$$\text{CORE MOIETY} \text{ —— } (R)_j$$

including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof, wherein j is an integer from one to three, the core moiety is non-cyclic or cyclic and R may be selected from among: hydrogen, halogen (preferably bromine, chlorine, fluorine and iodine), hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups, and formula I.

Preferred R substituents having a structure other than formula I include, but are not limited to, 2-bromopropyl, 4-chloropentyl, cyclohexyl, cyclopentyl, 3-dimethylaminobutyl, ethyl, hexyl, 2-hydroxyethyl, 5-hydroxyhexyl, 3-hydroxy-n-butyl, 3-hydroxypropyl, isobutyl, isopropyl, 2-methoxyethyl, 4-methoxy-n-butyl, methyl, n-butyl, n-propyl, phenyl, t-butyl and the like. Particularly preferred R, having a structure other than formula I, are ethyl, methyl, or hydrogen.

The inventive compounds have at least one R of the following formula I:

$$-(CH_2)_n-C-(R_1)_p \quad , \qquad I$$

wherein n is an integer from three to twenty; p is two or three; R_1 is selected from among hydrogen, halogen, hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group, =O, $-(CH_2)_s-C(R_2)_t$ (wherein s is zero or an integer from one to ten, t is two or three, R_2 is hydrogen, halogen, hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group, or =O); at least one R_1 or one R_2 is =O; and a second R_1 or second R_2, bonded to the same, respective -C as the at least one R_1 or one R_2, is other than =O or hydroxide. In the inventive compounds, when the core moiety is xanthine, and R corresponds to an unsubstituted, straight-chain ω-1 oxoalkyl, a total number of carbon atoms between the xanthine and the =O of R_1 or R_2 is not less than seven. Optionally, $(CH_2)_n$ and/or $(CH_2)_s$ may be substituted by a hydrogen, halogen, hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group.

Preferably, n is an integer from about eight to about eighteen, more preferably, an integer from about ten to about eighteen. In especially preferred compounds, p is two, one R_1 of $-C(R_1)_2$ is =O, and the second R_1 is hydrogen, a $C_{(1-10)}$ alkyl, a halo-substituted $C_{(1-10)}$ alkyl, and a substituted or unsubstituted $C_{(1-10)}$ alkoxyl.

Although other possible substituents are within the scope of the inventive compounds and pharmaceutical compositions thereof, representative, non-exhaustive substituents for the $R/R_1/R_2$ substituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyalkyl, $C_{(2-10)}$ alkenyl, cyclic and heterocyclic groups may be amido, amino, $C_{(2-8)}$ alkenyl, $C_{(1-8)}$ alkyl (including, *e.g.*, branched and unbranched alkyl or alkenyl groups), azido, carbonato, carbonyl, carboxyl, cyano, $C_{(1-8)}$ haloalkyl (including, *e.g.*, mono-, di- and tri-haloalkyl substituents, such as trihalomethyl), isocyanoto, isothiocyano, phospho, phosphonato, primary, secondary or tertiary hydroxyl (including, *e.g.*, any one of various diols, methanol, butanol, 1-cyclopentanol, ethanol, 2-ethyl-3-methyl-1-propanol, pentanol, propanol, and methylcyclohexanol), sulfonato, sulfonyl, sulfoxyl, thioamido, thiocarbonato, thionato, thiolnato, thiol, thioureido and ureido.

Representative $R/R_1/R_2$ cyclic groups may be, but are not limited to: anthracenyl, bicyclo[4.4.0]decanyl, bicyclo[2.2.1]heptanyl, bicyclo[3.2.0]heptanyl, bicyclo[4.1.0]heptanyl, bicylo[2.2.1]hexanyl, bicyclo[4.3.0]nonanyl, bicyclo[2.2.2]octanyl, biphenyl, cyclopentadienyl, cyclopentanyl, cyclobutanyl, cyclobutenyl, cycloheptanyl, cyclohexanyl, cyclooctanyl and cyclopropanyl, 1,2-diphenylethanyl, fluorenyl, indenyl, hydroxy, phenyl, quinonyl, terphenyl, napthalenyl, phenanthrenyl, terphenyl, toluenyl and

xylenyl. Due primarily to availability and ease of synthesis, more preferred $R/R_1/R_2$ cyclic groups include less complex ring systems, such as, for example, cyclopentanyl and cyclohexanyl, cyclopentadienyl, phenyl, indenyl, toluenyl and xylenyl.

$R/R_1/R_2$ heterocyclic groups may include azetidinyl, benzofuranyl, benzothiophenyl, carbazolyl, furanyl, glutarimidyl, indolyl, isoquinolinyl, oxazolyl, oxetanyl, oxiranyl, pyrrolidinyl, pyranyl, piperidinyl, pyridinyl, pyrrolyl, quinolinyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydrothiophenyl, thiophenyl, derivatives thereof and the like. Preferred $R/R_1/R_2$ heterocyclic groups are furanyl, indolyl, thyminyl and xanthinyl, although other heterocyclic groups are within the scope of the inventive compounds.

A non-cyclic core moiety may include, but is not limited to, for example, acetamido, amido, amino, amino acid (one or two), carboxyl, carbonyl, terminal halogen or hydrogen atom, hydroxyl, glutaric acid, glycinyl derivative, phospho, phosphonato, sulfato, sulfonato, sulfonyl, sulfoxyl, simple ionic functional group, thiol, thiolnato or the like. Exemplary core moiety amino acids may include one or more of the following: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. The non-cyclic core moiety may preferably be an amido, carboxyl, or hydroxyl group, a hydroatom, or a dipeptide comprising two amino acids selected from the foregoing exemplary list. A non-cyclic, halogen-core moiety may be, for example, bromine, chlorine, fluorine or iodine.

A cyclic core may be at least one five- to seven-member, non-heterocyclic ring or a heterocycle. The at least one five- to seven-membered cyclic core may preferably have from one to three, five- to six-membered ring structures in a predominantly planar configuration. For example, the core moiety may be selected from the group consisting of substituted or unsubstituted phenyl; biphenyl; cyclohexanyl; cyclohexanedionyl; cyclopentanedionyl; naphthalenyl; hydroxyphenyl; quinonyl; salicylic acid and derivatives thereof; stilbenyl, tricyclododecanyl or the like.

Although other heterocyclic cores are within the scope of the invention, the following representative compounds are preferred: substituted or unsubstituted barbituric acid; benzamido; glutarimidyl; homophthalimidyl; hydrophthalimidyl; imidazolyl; amido; indomethacinyl; isocarbostyrilyl; lumazinyl; N-alkylheterocyclic; N-heterocyclic; pteridinyl; phthalimidyl; piperidinyl; pyridinyl; pyrimidinyl; pyrrolyl; quaternized N-heterocyclic; quinolizinedionyl; quinazolinonyl; quinolinyl; recorsinol; succinimidyl; theobrominyl; thyminyl; triazinyl; uric acid, uracyl; vitamins A, E or K; or xanthinyl.

Preferably, R is bonded to a nitrogen of the core moiety, if present, most preferably to the nitrogen of a glutarimidyl, methylthyminyl, thyminyl, uracyl or xanthinyl core.

In representative, preferred compounds, R having formula I may be bonded to an N_1 nitrogen of glutarimidyl; N_1 nitrogen of xanthinyl (and N_3 and N_7 xanthinyl nitrogens may be independently substituted by a member selected from the group consisting of hydrogen, $C_{(1-6)}$ alkyl, fluoro, chloro and amino); N_3 nitrogen of a thyminyl or methylthyminyl; or N_1 nitrogen of uracyl. Alternatively, R having formula I may be bonded to N_1 and N_3 xanthinyl nitrogens and an N_7 xanthinyl nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino;

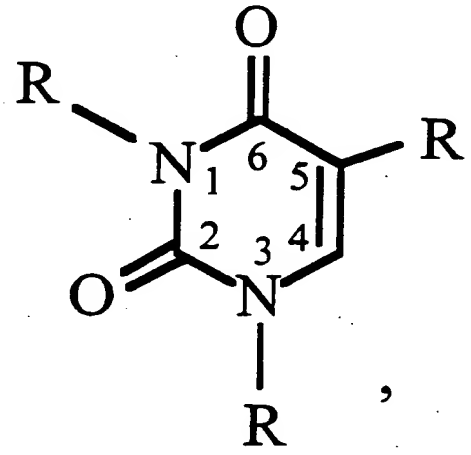
Representative substituents for the non-heterocyclic ring and heterocyclic cores correspond to the representative substituents previously defined for $R/R_1/R_2$ substituted cyclic or heterocyclic groups. Preferred non-heterocyclic ring cores include substituted or unsubstituted 1,3-cyclohexanedionyl; 1,3-cyclopentanedionyl; 1,3-dihydroxynaphthalenyl; orthohydroxyphenyl.

Preferred heterocyclic cores include substituted or unsubstituted glutarimidyl, methylthyminyl, methyluracyl, thyminyl, theobrominyl, uracyl and xanthinyl, most preferably halogen-substituted xanthinyl. Exemplary preferred cores include: $C_{(1-6)}$ alkyl-substituted thyminyl; $C_{(1-6)}$ alkyl-substituted uracyl; 1,3-dihydroxynapthalenyl; 3,3-dimethylglutarimidyl; dihydrothyminyl; 2,4-dioxohexahydro-1.3.5-tetrazinyl; hexahydrophthalimidyl; homophthalimidyl; 2-hydroxypyridine; β-ionone as vitamin A methylbarbituric acid; 2,6,6-methyl-1-cyclohexene-1-acetaldehyde as vitamin A; methyldihydroxypyrazolopyrimidinyl, specifically, 1,3-dimethyldihydroxypyrazolo[4,3-d]pyrimidinyl;1-methyl-5,6-dihydrouracyl; 1,7-dimethylxanthinyl, 3,7-dimethylxanthinyl; 7-methylhypoxanthinyl; 1-methyllumazinyl; 3-methyl-7-methylpivaloylxanthinyl; methylpyrrolopyrimidinyl; 1-methylpyrrolo [2,3-d] pyrimidinyl; 1-methyl-2,4(1H,3H)-quinolizinedionyl (1-methylbenzoyleneurea); methylthyminyl; 1-methyluracyl; 3-methylxanthinyl; orotic acid; prostacyclin; 1-pyrrole amido; 2-pyrrole amido; 3-pyrrole amido; quinazolin-4(3H)-onyl; 1,2,3,4-tetrahydroisoquinolonyl; tetrahydrophthalimidyl; sulindac; uracyl fused to naphthalene; 5- and/or 6-position substituted uracyl (such as, for example, 5-bromouracyl); and 8-substituted xanthinyl (having substituents such as N or S).

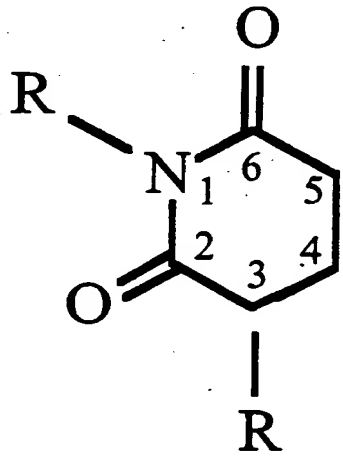
Preferably, R is bonded to a nitrogen of the core moiety, if present, most preferably to the nitrogen of a glutarimidyl, methylthyminyl, thyminyl, uracyl or xanthinyl core. In representative, preferred compounds, R having formula I may be bonded to an N_1 nitrogen of glutarimidyl; N_1 nitrogen of xanthinyl (and N_3 and N_7 xanthinyl nitrogens may be independently substituted by a member selected from the group consisting of hydrogen, $C_{(1-6)}$ alkyl, fluoro, chloro and amino); N_3 nitrogen of a thyminyl or methylthyminyl; or N_1 nitrogen of uracyl. Alternatively, R having formula I may be bonded to N_1 and N_3 xanthinyl nitrogens and an N_7

xanthinyl nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino.

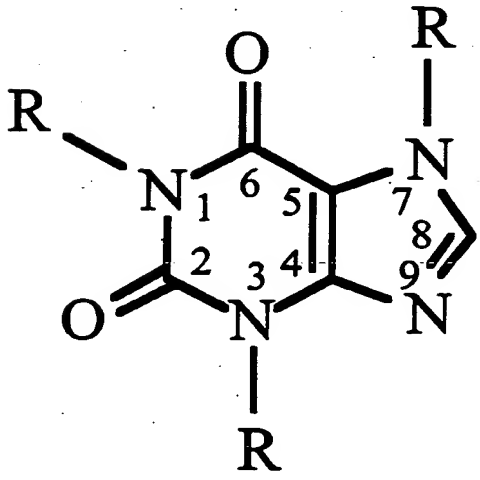
Representative, preferred inventive compounds are compounds of formulas II, III or IV:



II ,



III ,



IV ,

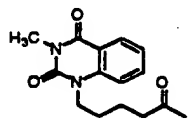
wherein R is defined above.

The invention also provides a pharmaceutical composition. Pharmaceutical compositions of the inventive compounds comprise a pharmaceutical carrier, diluent or excipient and some amount of an inventive compound. The compound may be present in an amount to effect a physiological response, or it may be present in a lesser amount such that the user will need to take two or more units of the composition to effect the treatment intended. These compositions may be made up as a solid or liquid or in a gaseous form. Or one of these three forms may be transformed to another at the time of being administered such as when a solid is delivered by aerosol means, or when a liquid is delivered as a spray or aerosol.

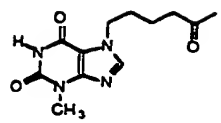
The nature of the composition and the pharmaceutical carrier, diluent or excipient may, of course, depend upon the intended route of administration, for example, parenterally, topically, orally or by inhalation for treatment of a patient with disease symptoms. For topical administration, the pharmaceutical composition may be in the form of a cream, ointment, liniment, lotion, pastes, aerosols and drops suitable for administration to the skin, eye, ear or nose. For parenteral administration, the pharmaceutical composition may be in the form of a steril injectable liquid such as an ampule or an aqueous or non-acqueous liquid suspension. For oral administration, the pharmaceutical composition may be in the form of a tablet, capsule, powder, pellet, atroche, lozenge, syrup, liquid or emulsion.

The invention includes a method for treating an individual having a variety of diseases. The disease is characterized by or can be treated by inhibiting an immune response or a cellular response to external or *in situ* primary stimuli. Treatment of the disease states involves mediating the cellular response through a specific phospholipid-based second messenger acting adjacent to a cell membrane inner leaflet. The second messenger pathway is activated in response to various noxious or proliferative stimuli, characteristic of disease states treatable using the inventive compounds or pharmaceutical compositions thereof. Biochemistry of this second messenger pathway is described herein. More specifically, the invention includes methods for treating or preventing clinical symptoms of various disease states or reducing toxicity of other treatments by inhibiting cellular signaling through a second messenger pathway involving signaling through phosphatidic acid and through glycan phosphatidylinostinol (Gly PI).

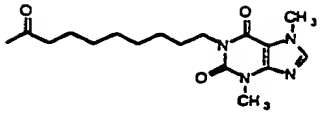
Illustrative, non-limiting, examples of compounds of the invention include the following:

1208 1-(5-Oxohexyl)-3-methylbenzoyleneurea 

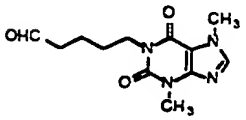
1437 7-(5-Oxohexyl)-3-methylxanthine



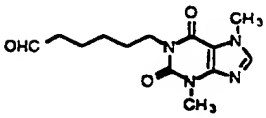
1516 1-(9-Oxodecyl)-3,7-dimethylxanthine



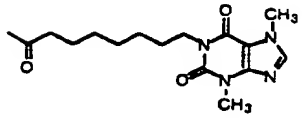
1526 1-(5-Oxopentyl)-3,7-dimethylxanthine



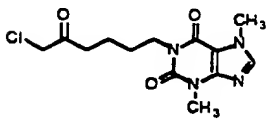
1549 1-(6-Oxohexyl)-3,7-dimethylxanthine



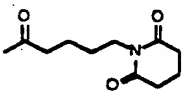
1568 1-(8-Oxononyl)-3,7-dimethylxanthine



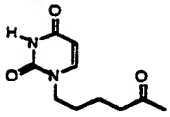
1595 1-(6-Chloro-5-oxohexyl)-3,7-dimethylxanthine



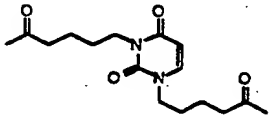
1602 N-(5-Oxohexyl)glutarimide



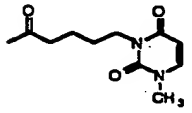
1803 1-(5-Oxohexyl)uracil



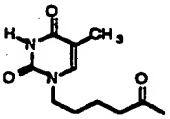
1805 1,3-Bis-(5-Oxohexyl)uracil



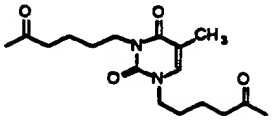
1810 3-(5-Oxohexyl)-1-methyluracil



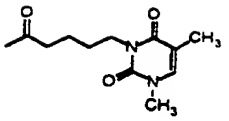
1901 1-(5-Oxohexyl)thymine



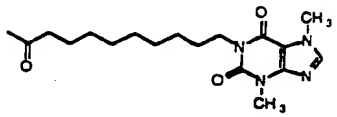
1902 1,3-Bis-(5-Oxohexyl)thymine



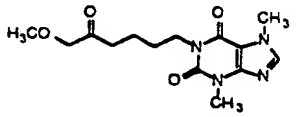
1909 3-(5-Oxohexyl)-1-methylthymine



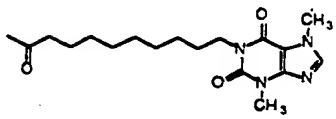
2510 1-(10-Oxoundecyl)-3,7-dimethylxanthine



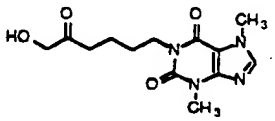
2521 1-(6-Methoxy-5-Oxohexyl)-3,7-dimethylxanthine



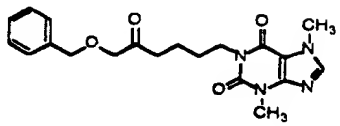
2524 1-(11-Oxododecyl)-3,7-dimethylxanthine



2543 1-(6-Hydroxy-5-oxohexyl)-3,7-dimethylxanthine



2572 1-(6-Benzoyloxy-5-oxohexyl)-3,7-dimethylxanthine



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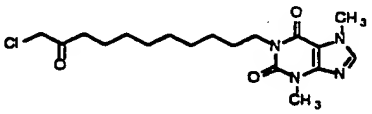

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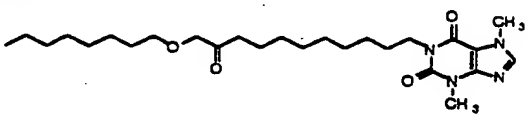

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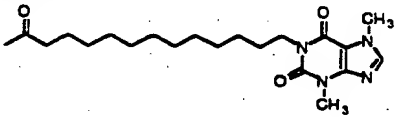
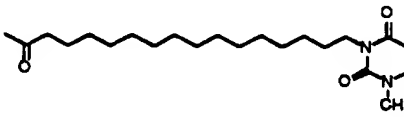
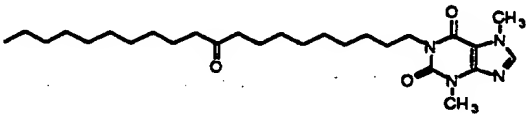


3519 1-(11-Oxoheptadecyl)-3,7-dimethylxanthine



3525 1-(11-Oxoeicosyl)-3,7-dimethylxanthine



Synthesis of the Inventive Compounds

The invention includes methods for preparing compounds according to the invention. Exemplary methods for preparing the inventive compounds are discussed below and in the following examples.

In a method according to the invention, a compound containing a desired core (intended as a "core moiety" in compounds of the invention) undergoes a reaction to produce an anion. The anion is then subsequently reacted with a substituted olefin to displace a targeted functional group on the olefin, resulting in an intermediate olefinic product. In a preliminary reaction, a predetermined amount of a core-containing compound is reacted with a suitable base, a solvent and a substituted olefin, the substituted olefin having at least one functional group which may be substituted in a displacement reaction by the desired core-containing compound.

Preferred bases include, but are not limited to, sodium hydride, sodium amide, sodium alkoxide, lithium hydride, potassium hydride, lithium amide, sodium amide and potassium amide. An especially preferred base is sodium hydride. Preferred solvents may be dimethylsulfoxide, dimethylformamide, or an alcohol. Exemplary preferred alcohols include, but are not limited to, methanol, ethanol or isopropanol. Any substituted olefin comprising a chain structure of the inventive compounds may be used in the preliminary reaction according to the invention. Preferred olefins may be ω-substituted olefins. Preferred substituted olefins include, but are not limited to halo-substituted olefins.

Generally, an intermediate olefinic product may be converted to a primary or secondary alcohol and then to the corresponding aldehyde- or ketone-containing inventive compound. Although other methods are within the scope of the invention, the following are possible methods for obtaining the inventive compounds.

In one synthetic protocol, the intermediate olefinic product, having a composite structure of the core-containing compound and substituted olefin may subsequently be converted to a corresponding compound having a primary hydroxyl functional group. The intermediate olefinic product is reacted with a hydroborating agent to obtain a desired borane derivative. The borane derivative is subsequently reacted in an oxidative hydrolysis reaction with an oxidative-hydrolyzing agent to obtain the corresponding compound having the desired primary hydroxyl functional group. Exemplary hydroborating agents include, but are not limited to, diborane, borane-methyl sulfide complex, borane-pyridine complex, hexylborane, diamylborane, and 9-borabicyclo[3.3.1]no-nane, most preferably, borane-tetrahydrofuran complex. Exemplary oxidative-hydrolyzing agents include strong oxidizers such as a hydrogen peroxide solution and the like. These primary hydroxyl functional groups may then be convered to the desired aldehyde.

In a synthetic protocol for preparing inventive ketones, the intermediate olefinic product may be converted to a corresponding secondary hydroxy functional group by first obtaining a diol, then converting the diol to a haloester and preparing an intermediate epoxide product, which is subsequently reacted to form a desired secondary alcohol intermediate.

In one synthesis, the intermediate olefinic product is reacted with a suitable oxidizing agent. Preferred oxidixing agents include, but are not limited to, osmium tetroxide. Preferred oxidizing agents, such as osmium tetroxide may require a catalytic amount of the oxidizing agent in the presence of a regenerating agent. Exemplary, regenerating agents may be 4-methylmorpholine-N-oxide and trimethylamine-N-oxide. An especially preferred regenerating agent is 4-methylmorpholine-N-oxide. In a subsequent halogenation reaction, the resulting diol is converted to a haloester using a halogenating agent in the presence of an organic acid. Exemplary halogenating agents include hydrogen bromide and hydrogen chloride. Preferred organic acids may be acetic acid and propionic acid. The resulting haloester is subsequently reacted with a basic ester-hydrolyzing reagent to obtain a desired intermediate epoxide product. Preferred ester-hydrolyzing agents include, but are not limited to metal alkoxides and metal hydroxides. Especially preferred metal alkoxides are sodium methoxide, ethoxide, isopropoxide and pentoxide. A preferred metal hydroxide is sodium hydroxide.

In an alternative synthesis, the intermediate olefinic product may be reacted with an organic peracid to obtain a desired intermediate epoxide product directly. Preferred exemplary organic peracids include 3-chloroperoxybenzoic acid, peracetic acid and trifluoroperacetic acid. An especially preferred peracid is 3-chloroperoxybenzoic acid.

Subsequently, the corresponding intermediate epoxide product, which may be prepared by other methods than those discussed above, is reacted with a reducing agent to convert the correponding epoxide to the intermediate secondary alcohol product. Exemplary reducing agents may be selected from the non-exhaustive group of sodium borohydride or lithium aluminum hydride, and hydrogen gas in the presence of a metal catalyst. Preferred metal catalysts may be, for example, palladium, platinum, or Raney nickel.

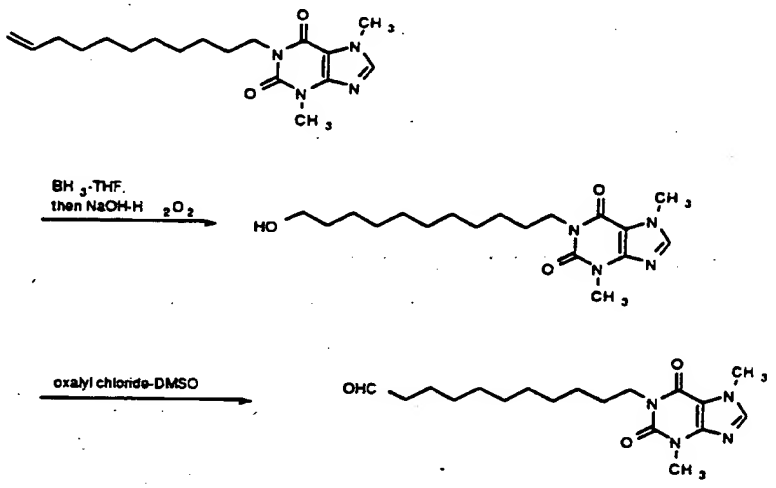
The intermediate primary or secondary alcohol product may also be prepared by by reacting a compound having at least one hydroxyl group with a predetermined amount of a compound containing a desired core (intended as a "core moiety" in compounds of the invention) with a suitable base and a solvent. The compound having at least one hydroxyl group has at least one other functional group which may be substituted in a displacement reaction by the core-containing compound. Other functional groups may be, for example, halogen atoms.

In a final synthetic step, the intermediate primary or secondary alcohol product is reacted to obtain the desired inventive aldehyde- or keton-containing compound. In one synthetic procedure, the intermediate primary or secondary alcohol product is reacted with a suitable oxidizing agent, converting the hydroxyl functional group to the corresponding aldehyde

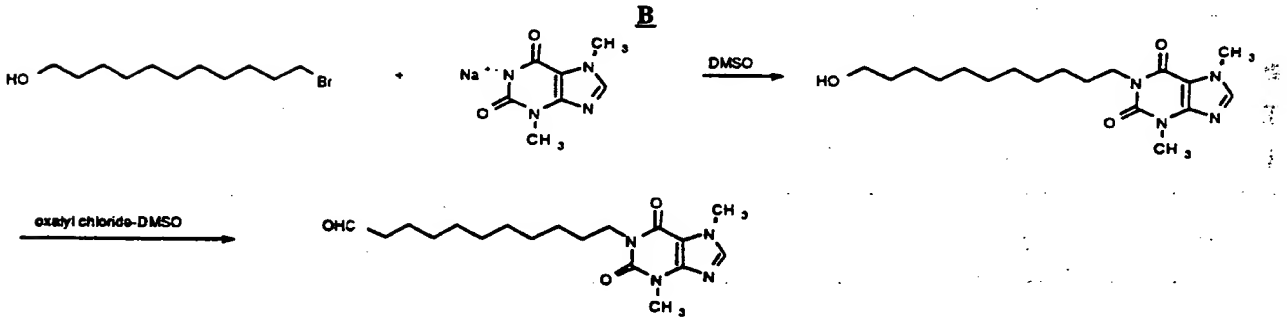
or ketone functional group. Exemplary oxidizing agents include, but are not limited to, oxalyl chloride-dimethylsulfoxide and other dimethylsulfoxide based oxidizing agents, and pyridinium dichromate and other chromium based oxidizing agents.

Schematic illustrations of the foregoing, representative processes for preparing an inventive aldehyde-containing compound from an olefinic intermediate or from a primary alcohol and compound containing a desired core are shown in schematics A and B, respectively:

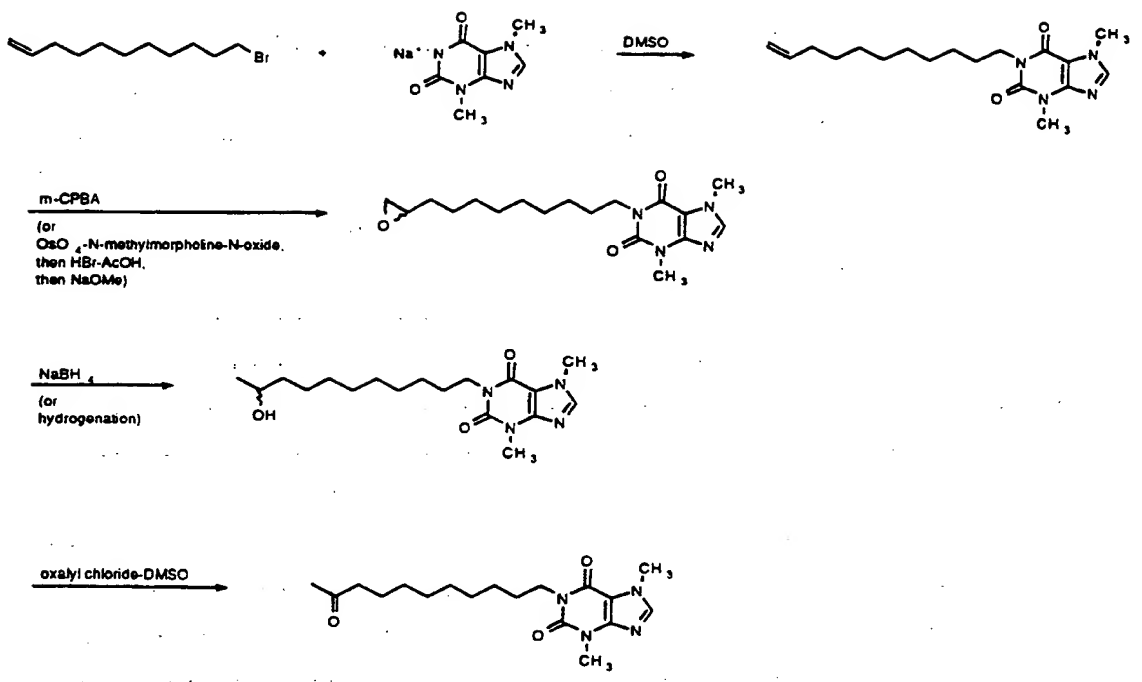
A



B



A schematic illustration of the foregoing, representative processes for preparing an inventive ketone-containing compound from an olefinic intermediate is shown in schematic C below:



Uses of the Invention Compounds and Pharmaceutical Formulations

The inventive compounds provide a method for maintaining homeostasis in cells contacted by primary stimuli by mitigating the effects of these primary stimuli on the secondary signaling pathways invoked within seconds of a primary stimulus, thereby providing a method for treating an individual having a variety of diseases. The disease is characterized by or can be treated by inhibiting an immune response or a cellular response to external or *in situ* primary stimuli. Treatment of the disease states involves mediating the cellular response through a specific phospholipid-based second messenger acting adjacent to a cell membrane inner leaflet. The second messenger pathway is activated in response to various noxious or proliferative stimuli, characteristic of disease states treatable using the inventive compounds or pharmaceutical compositions thereof. Biochemistry of this second messenger pathway is described herein. More specifically, the invention includes methods for treating or preventing clinical symptoms of various disease states or reducing toxicity of other treatments by inhibiting cellular signaling through a second messenger pathway involving signaling through phosphatidic acid and through glycan phosphatidylinostinol (Gly PI).

Gly PI consists of a phosphatidylinositol-1-phosphate (PIP) bound through the carbon 6-hydroxyl to a glucosamine residue, which in turn is bound, usually to 2-5 other glycan residues (1→4 type, linear bonds) containing an additional one to three phosphoethanolamine moieties, the last of which may be bound to an external protein such as Thy-1. Evidence suggests a broad variety of structural variation in the sn-1 and sn-2 positions of the glycerol/lipid

moiety of the phosphatidylinositol, as well as fatty acyl addition to the 2-OH group of the inositol. Several functional parameters of structure have been observed, the most remarkable of which point to a minimum presence of at least one myristoyl sidechain in Gly-PI molecules, the presence of both alkyl (ether) and acyl chains in the sn-1 position, and the presence of palmitate (C16:0) in the 2-OH position of the inositol in protein-binding Gly-PI. Thomas et al., *Biochemistry* (1991): 29: 5413-5422.

Recent research has demonstrated that 2-OH-acylation of the inositol moiety conveys resistance to hydrolysis with Gly PI-directed phospholipase C (P_iG-PLC, a phosphodiesterase which hydrolyzes Gly PI to glycan inositol phosphate and diacylglycerol) but not to Gly PI-directed phospholipase D (P_iG-PLD, a phosphodiesterase which hydrolyzes Gly PI to glycan inositol + phosphatidic acid).

Research has identified two functions of Gly-PI: 1) external protein binding, the purpose of which may be simple binding to the cell membrane or placement of conformational constraints on the structure of externally bound membrane proteins (*e.g.*, so that a particular portion of the molecule faces an extracellular environment); and 2) signal transduction, including part of the intracellular signal sent by insulin and a detectable portion of the signal transduced by Interleukin-2 (IL-2). We have found that signal transducing Gly-PI in B lymphocytes is hydrolyzed following anti-mu crosslinking, and then resynthesized rapidly. In these systems, two Gly-PI species are synthesized: a) $GlyPI_1$, containing 1-myristoyl 2-palmitoyl, 1-o-tetradecanyl (myristyl) 2-palmitoyl and 1-myristyl 2-myristyl phosphatidylinositol; and b) Gly PI_2, containing 1-myristoyl 2-oleoyl and 1-o-myristyl 2-linoleoyl phosphatidylinositol. Fraction (a) above contains a 1:1 mole content of C22 or C20 acyl groups attached to the inositol phosphate. The Gly-PI_1 fraction, identified by glucosamine labeling followed by mass spectrometry, exhibits a characteristic tripartite peak (glycan-inositol: 2-OH-acyl: phosphatidic acid moieties) and is uniformly inositol 2-OH acylated. Therefore, fraction (a) conveys resistance to P_iG-PLC but not to P_iG-PLD, suggesting that the observed fraction, when hydrolyzed, will generate 1-myristyl and 1-o-myristyl phosphatidic acid species, subsequently observed.

Thus, inventive compounds, useful in treating diseases and reducing toxicity of other disease treatments, would affect cellular signaling through a second messenger pathway by interacting with binding and/or signaling functions of Gly PI.

For example, administration of an inventive compound *in vivo* or *ex vivo* provides a method to modify cellular behavior, the method comprising contacting cells (*in vivo* or *ex vivo*), whose behavior is to be modified, with an effective amount of an inventive compound or a pharmaceutical composition thereof. The inventive methods: (1) inhibit proliferation of tumor cells; (2) suppress activation of T-cells by antigen or IL-2 stimulation; (3) suppress activation of

monocyte/macrophage cells by endotoxin, TNF, IL-1 or GM-CSF stimulation; (4) suppress antibody production of B-cells in response to an antigen, IL-4 or CD40 ligand; (5) inhibit the proliferation of smooth muscle cells in response to growth factors capable of stimulating said proliferation; (6) lower systemic vascular resistance conferred by endothelial cells; (7) lower systemic vascular resistance induced by endothelial cells; (8) lower expression of adhesion molecules induced by enhancers thereof; (9) suppress the activation of T-cells and macrophages by HIV; (10) inhibit the proliferation of kidney mesangial cells in response to stimulation by IL-1 and/or MIP-1α and/or PDGF and/or FGF; (11) enhance the resistance of kidney glomerular or tubular cells to cyclosporin A or amphotericin B; (12) prevent the release of MIP-1α by IL-1, TNF, or endotoxin stimulated monocytes and macrophages; (13) prevent the release of platelet activating factor by IL-1, TNF, or endotoxin treated megakaryocytes, fibroblastic cells, and macrophages; (14) prevent the down-regulation of receptors for cytokines in TNF-treated hematopoietic progenitor cells; (15) suppress the production of metalloproteases in IL-1-stimulated or TNF-stimulated glomerular epithelial cells or synovial cells, being; (16) enhance the resistance of gastrointestinal or pulmonary epithelial cells to cytotoxic drugs or radiation; (17) enhance the antitumor effect of a non-alkylating antitumor agent; (18) to inhibit the production of osteoclast activating factor in response to IL-1; (19) inhibit degranulation in response to IgE; (20) enhance the release of adrenergic neural transmitters, dopamine, norepinephrine, or epinephrine, or the neurotransmitter, acetylcholine; (21) modulate the post-synaptic "slow current" effects of the adrenergic neurotransmitters dopamine, epinephrine, or norepinephrine, or the neurotransmitter acetylcholine; (22) suppress signaling by neurotransmitters including acetyl choline, leuenkephalin and seretonin; or (23) increase seizure theshold.

A disease state or treatment-induced toxicity is selected from the group consisting of: tumor progression involving tumor stimulation of blood supply (angiogenesis) by production of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF); tumor invasion and formation of metastases through adhesion molecule binding, expressed by vascular endothelial cells (VCAM and ICAM); tissue invasion through tumor metalloprotease production such as MMP-9; autoimmune diseases caused by dysregulation of the T cell or B cell immune systems, treatable by suppression of the T cell or B cell responses; acute allergic reactions including, but not limited to, asthma and chronic inflammatory diseases, mediated by pro-inflammatory cytokines including tumor necrosis factor (TNF) and IL-1, and rheumatoid arthritis, osteoarthritis, multiple sclerosis or insulin dependent diabetes mellitus (IDDM), associated with enhanced localization of inflammatory cells and release of inflammatory cytokines and metalloproteases; smooth muscle cell, endothelial cell, fibroblast and other cell type proliferation in response to growth factors, such as PDGF-AA, BB,

FGF, EGF, etc. (*i.e.*, atherosclerosis, restenosis, stroke, and coronary artery disease); activation of human immunodeficiency virus infection (AIDS and AIDS related complex); HIV-associated dementia; kidney mesangial cell proliferation in response to IL-1, MIP-1α, PDGF or FGF; inflammation; kidney glomerular or tubular toxicity in response to cyclosporin A or amphotericin B treatment; organ toxicity (*e.g.*, gastrointestinal or pulmonary epithelial) in response to a cytotoxic therapy (*e.g.*, cytotoxic drug or radiation); effects of non-alkylating anti-tumor agents; inflammation in response to inflammatory stimuli (*e.g.*, TNF, IL-1 and the like) characterized by production of metalloproteases or allergies due to degranulation of mast cells and basophils in response to IgE or RANTES; bone diseases caused by overproduction of osteoclast-activating factor (OAF) by osteoclasts; CNS diseases resulting from over-stimulation by pro-inflammatory neurotransmitters such as, acetylcholine, serotonin, leuenkephalin or glutamate; acute inflammatory diseases such as septic shock, adult respiratory distress syndrome; multi-organ dysfunction associated with inflammatory cytokine cascade; and combinations thereof.

Indications useful for administering compounds of the invention include, but are not limited to: a hormone-related disorder, an autoimmune disease, inflammation, coronary artery disease, hypertension, unwanted immune response (such as allograft reactions), viral infection, nephritis, mucositis, and various allergic responses. Allergic responses include acute allergic response and thus rhinorrhea, sinus drainage, diffuse tissue edema, and generalized pruritus. As well as the following, other chronic allergic responses include, dizziness, diarrhea, tissue hyperemia, and lacrimal swelling with localized lymphocyte infiltration. Allergic reactions are also associated with leukotriene release and the distal effects thereof, including asthmatic symptoms (*e.g.*, development of airway obstruction, a decrease in FEV1, changes in vital capacity, and extensive mucus production).

In a large number of cells, signaling is dependent upon generation of a broad variety of PA species, some of which are generated from lyso-PA by the enzyme lyso-PA acyl transferase and some of which are generated from 2-O-acyl glycan-PI by PiG-PLD. Generation of each of these PA species (the predominant forms being: 1-acyl and 1-alkyl 2-linoleoyl PA compounds, generated by LPAAT; and 1-myristyl 2-palmitoyl and 1-o-myristyl 2-palmitoyl, generated by PiG-PLD) serves to effect both proliferative and/or inflammatory signaling in the diseases discussed and cell systems described above.

The inventive compounds are of particular significance for inhibiting IL-2-induced proliferative response. IL-2 signaling inhibition is potentially useful in the treatment of numerous disease states involving T-cell activation and hyperproliferation. Exemplary autoimmune diseases treated by inhibiting IL-2 signaling are lupus, scleroderma, rheumatoid

arthritis, multiple sclerosis, glomerula nephritis as well as potential malignancies, including but not limited to, chronic myelogenous leukemia as well as others.

The compounds of the invention further are able to decrease enhanced levels of a relevant PA and DAG resulting from stimulation of synaptosomes with acetylcholine and/or epinephrine. This suggests that the effects of the compounds of the invention are to both enhance the release of inhibitory neural transmitters such as dopamine, and to modulate the distal "slow current" effects of such neurotransmitters.

Thus, the drugs of the invention are also useful to raise the seizure threshold, to stabilize synapses against neurotoxins such as strychnine, to potentiate the effect of anti-Parkinson drugs such as L-dopa, to potentiate the effects of soporific compounds, to relieve motion disorders resulting from administration of tranquilizers, and to diminish or prevent neuron overfiring associated with progressive neural death following cerebral vascular events such as stroke. In addition, the compounds of the invention are useful in the treatment of norepinephrine-deficient depression and depressions associated with the release of endogenous glucocorticoids, to prevent the toxicity to the central nervous system of dexamethasone or methylprednisolone, and to treat chronic pain without addiction to the drug. Further, the compounds of the invention are useful in the treatment of children with learning and attention deficits and generally improve memory in subjects with organic deficits, including Alzheimer's patients.

While dosage values will vary, therapeutic efficacy is achieved when the compounds of the invention are administered to a human subject requiring such treatment as an effective oral, parenteral, or intravenous sublethal dose of about 50 mg to about 5000 mg per day, depending upon the weight of the patient. A particularly preferred regimen for use in treating leukemia is 4-50 mg/kg body weight. It is to be understood, however, that for any particular subject, specific dosage regimens should be adjusted to the individual's need and to the professional judgment of the person administering or supervising the administration of the inventive compounds.

Pharmaceutical Formulations

A suitable formulation will depend on the nature of the disorder to be treated, the nature of the medicament chosen, and the judgment of the attending physician. In general, the inventive compounds are formulated either for injection or oral administration, although other modes of administration such as transmucosal or transdermal routes may be employed. Suitable formulations for these compounds can be found, for example, in *Remington's Pharmaceutical Sciences* (latest edition), Mack Publishing Company, Easton, PA.

The inventive compounds and their pharmaceutically acceptable salts can be employed in a wide variety of pharmaceutical forms. The preparation of a pharmaceutically acceptable salt will be determined by the chemical nature of the compound itself, and can be prepared by conventional techniques readily available. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25 mg to about 1 gram, wherein the amount of inventive compound per dose will vary from about 25 mg to about 1 gram for an adult. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension. Where the inventive composition is in the form of a capsule, any routine encapsulation is suitable, for example, using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule, any pharmaceutical carrier routinely used for preparing dispersions of suspensions may be considered, for example, aqueous gums, celluloses, silicates or oils and are incorporated in a soft gelatin capsule shell. A syrup formulation will generally consist of a suspension or solution of the compound or salt thereof in a liquid carrier (*e.g.*, ethanol, polyethylene glycol, coconut oil, glycerine or water) with a flavor or coloring agent.

The amount of inventive compound required for therapeutic effect on topical administration will, of course, vary with the compound chosen, the nature and severity of the disease and the discretion of the treatment provider. Parenteral includes intravenous, intramuscular, subcutaneous, intranasal, intrarectal, intravaginal or intraperitoneal administration. Appropriate dosage forms for such administration may be prepared by conventional techniques. A typical parenteral composition consists of a solution or suspension of the inventive compound or a salt thereof in a sterile or non-aqueous carrier, optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil, or sesame oil. The daily dosage for treatment of sepsis or another severe inflammatory condition via parenteral administration is suitable from about 0.001 mg/kg to about 40 mg/kg, preferably from about 0.01 mg/kg to about 20 mg/kg of an inventive compound or a pharmaceutically acceptable salt thereof calculated as the free base.

The inventive compounds may be administered orally. The daily dosage regimen for oral administration is suitably from about 0.1 mg/kg to about 1000 mg/kg per day. For administration the dosage is suitably from about 0.001 mg/kg to about 40 mg/kg of the inventive compound or a pharmaceutically acceptable salt thereof, calculated as the free base. The active ingredient may be administered from 1 to 6 times a day, sufficient to exhibit activity.

The inventive compounds may be administered by inhalation (*e.g.*, intranasal or oral) Appropriate dosage forms include an aerosol or a metered dose inhaler, as prepared by

conventional techniques. The daily dosage is suitably from about 0.001 mg/kg to about 40 mg/kg of the inventive compound or a pharmaceutically acceptable salt thereof, calculated as the free base. Typical compounds for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant.

The invention is illustrated by the following examples which should not be regarded as limiting the invention in any way.

Example 1

This example is a synthesis for inventive compound no. 1549 (see above for chemical name and structure). A mixture of 1.0 g of theobromine (5.5 mmol), available from Sigma, and 264 mg of 50% sodium hydride (5.5 mmol) in oil and 20 ml of dimethylsulfoxide was stirred for 50 minutes and then 1.0 g of 6-bromo-1-hexanol (5.5 mmol), available from (Aldrich), was added to the stirring mixture. After an additional 18 hours of stirring, the solution was treated with 50 ml of water and then extracted with two 25 ml aliqouts of hexane. The aqueous phase was extracted with three 35 ml aliquots of 25% ethanol-dichloromethane. The combined ethanol-dichloromethane extracts were dried over magnesium sulfate and then the solvent was evaporated under vacuum. Remaining dimethylsulfoxide was removed by distillation under a full pump vacuum, leaving 1.4 g of a white powder, 1-(6-hydroxyhexyl)-3,7-dimethylxanthine (5.0 mmol, 91% yield).

156 ml (172 mg) of dimethyl sulfoxide (2.2 mmol) were slowly added to a solution of 103 ml (150 mg) of oxalyl chloride (1.2 mmol) in dichloromethane at - 78 °C. A solution of 300 mg of 1-(6-hydroxyhexyl)-3,7-dimethylxanthine (1.1 mmol) in 5 ml of dichloromethane was added to the previous reaction mixture, followed by 15 minutes of stirring. The cold bath was removed after addition of 765 ml (555 mg) of triethylamine (5.5 mmol). At ambient termperature, the reaction was added to 20 ml water and extracted with three 50 ml aliquots of methylene chloride. The combined organic layers were washed with 20 ml of 1% aqueous hydrogen chloride, 20 ml of saturated aqueous sodium bicarbonate, and 20 ml of saturated aqueous salt solution, and then dried over sodium sulfate. Evaporating the solvent and recrystallizating an evaporation residue in chloroform/petroleum ether resulted in 267 mg of compound no. 1549 (87% yield).

Example 2

This example illustrates a synthesis for inventive compound no. 1595 (see above for chemical name and structure). 11.9 g of theobromine (66 mmol), available from Sigma, were added to a mixture of 10.7 g of bromohexene (66 mmol), available from Aldrich, and 1.58 g of sodium hydride (66 mmol) in 100 ml of dimethylsulfoxide and the resulting reaction mixture stirred for 43 hours. The reaction mixture was treated with 200 ml of water and then extracted

with three 80 ml aliquots of dichloromethane. The combined extracts were washed three times with 100 ml of water per wash and subsequently dried over magnesium sulfate. The solvent was evaporated under vacuum, leaving 17 g of white powder, 1-(5-hexenyl)-3,7-dimethylxanthine (65 mmol, 98% yield).

Six drops of 2.5% osmium tetraoxide in *t*-butanol were added to a mixture of 1.07 g of 1-(5-hexenyl)-3,7-dimethylxanthine (4.1 mmol) , prepared above, and 1.44 g of *N*-methylmorpholine-*N*-oxide (12.3 mmol) in 20 ml of water and 10 ml of acetone. After stirring the resulting reaction mixture for 48 hours, it was treated with 20 ml of 20% aqueous sodium dithionite solution. After 2 minutes, the mixture was extracted with three 30 ml aliquots of 25% ethanol-dichloromethane solution. The combined extracts were dried over magnesium sulfate and the solvents were evaporated under vacuum, leaving 750 mg of white powder, 1-(5,6-dihydroxyhexyl)-3,7-dimethylxanthine (2.53 mmol, 62% yield).

3.4 ml of 30% hydrogen bromide-acetic acid were added (over a 30 second period) to 1.0 g of 1-(5,6-dihydroxyhexyl)-3,7-dimethylxanthine (3.38mmol). The resulting mixture was stirred 2.5 hours until all solid had dissolved. The solution was poured carefully over a mixture of 12 g of sodium bicarbonate and 50 ml of ice water. After carbon dioxide evolution subsided, the mixture was extracted with three 25 ml aliquots of dichloromethane. The combined extracts were dried over magnesium sulfate and the solvent was evaporated under vacuum to obtain 1.3 g of 1-(5-acetoxy-6-bromohexyl)-3,7-dimethylxanthine (3.24 mmol, 96% yield), as a viscous oil dissolved in 5 ml of methanol. Subsequently, 3.9 ml of a 1*M* sodium methoxide in methanol solution was added over 30 seconds. After stirring for 20 minutes, the solution was treated with 20 ml of water and then extracted with three 15 ml aliquots of dichloromethane. The combined extracts were dried over magnesium sulfate and solvents evaporated under vacuum, leaving 900 mg of white crystals, 1-(5,6-oxidohexyl)-3,7-dimethylxanthine (3.24 mmol, 100% yield).

0.634 g of Oxalyl chloride (5.00 mmol) were added to a solution of 0.390 g of dimethylsulfoxide (5.00 mmol) in 20 ml of dichloromethane at -60 °C. The resulting mixture was stirred for 5 minutes. 0.548 g of 1-(5,6-oxidohexyl)-3,7-dimethylxanthine (2.00 mmol), prepared above, and 0.0064 g of methanol (0.20 mmol) were added to the solution and the resulting reaction mixture was stirred for a further 30 minutes at -60 °C. 1.01 g of Triethylamine (10.0 mmol) were added and the mixture stirred at -60 °C for an additional 10 minutes before warming the mixture to 25 °C over 30 minutes. The reaction was washed with 30 ml of saturated ammonium chloride solution, 30 ml of water and 30 ml of brine. The organic phase was dried over magnesium sulfate and the solvent evaporated, yielding crude product purified by chromatography using silica and a methanol/dichloromethane eluant to obtain 0.38 g of compound no. 1595 (62% yield).

Example 3

This example is a synthesis for inventive compound no. 1810. 398 mg of sodium hydride (15.9 mmol) were added to a stirring solution of 2.0 g of 1-methyluracil (15.9 mmol) in 40 ml of dimethylsulfoxide. After 15 minutes, 2.85 g of 6-bromo-2-hexanone (15.9 mmol) were added and the resulting reaction mixture stirred for 4 days. The reaction was then poured into 80 ml water and extracted with four 50 ml aliquots of dichloromethane. The organic layers were combined, washed with 20 ml of aqueous saturated salt solution and dried over sodium sulfate. The solvent was evaporated under vacuum, leaving a brown oil. The crude product was purified by column chromatography using silica and a 20% petroleum ether/ethyl acetate eluant, yielding 1.79 mg of compound no. 1810 (50% yield).

Example 4

This example is a synthesis of inventive compound no. 1909 (see above for chemical name and structure). A solution of 1.00 g of 1-methylthymine (7.1 mmol), available from Aldrich, and 171 mg of sodium hydride (7.1 mmol) in 20 ml of dimethylsulfoxide (20 ml) was stirred at ambient temperature. After 45 minutes, 1.30 g of 6-bromo-2-hexanone (7.1 mmol) were added and the resulting reaction mixture was stirred for 72 hours. The reaction was poured into 50 ml of water and extracted with four 50 ml aliquots of dichloromethane. The organic layers were combined and washed with two 30 ml aliqouts of saturated aqueous salt solution and dried over magnesium sulfate. Evaporating the solvent left a brownish solid which upon recrystallization in dichloromethane/petroleum ether yielded 960 mg of a white solid, compound no. 1909 (56% yield).

Example 5

This example is a method of synthesis for inventive compound no. 2594. 1.26 g of 95% sodium hydride(50 mmol) were added to a solution of 7.2 g of theobromine (40 mmol) in 300 ml of dimethylsulfoxide. After 20 minutes of stirring, 7.95 g of undecenylmesylate (30 mmol) were added and the resulting mixture stirred for 12 hours at room temperature. The reaction was warmed to 70-80 °C and stirred for 4 hours and was then poured into a separatory funnel containing 1 L of water and extracted with five 200 ml aliquots of dichloromethane. The organic extracts were combined, washed with 100 ml of water and 100 ml of brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product obtained was further purified by flash chromatography over silica gel using a 20% hexane/dichloromethane eluant, producing 4.6 g of 1-(10-undecenyl)-3,7-dimethylxanthine (46.3% yield).

A solution of 4.3 g of 1-(10-undecenyl)-3,7-dimethylxanthine (13 mmol), 1.942 g of 4-methylmorpholine-N-oxide (16.6 mmol) and 9.5 mg of potassium osmate dihydrate (0.026 mmol) in 45 ml of acetone and 10 ml of water was stirred for 6 hours. A solution of 12 ml of 20% aqueous sodium sulphite was added and the resulting mixture stirred for 30 minutes. The reaction mixture was extracted with four 100 ml aliquots of 20% ethanol/dichloromethane. The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash chromatography over silica gel using a methanol (5%)/dichloromethane eluant, producing 3.6 g of 1-(10,11-dihydroxyundecanyl)-3,7-dimethylxanthine (76% yield).

3.6 g of 1-(10,11-dihydroxyundecanyl)-3,7-dimethylxanthine (10 mmol) were stirred with 6.2 ml of hydrogen bromide (8.4 g of a 30% solution in acetic acid, 31.1 mmol) for 90 minutes. The mixture was then added to a flask containing 100 ml of aqueous sodium bicarbonate solution and 75 ml of dichloromethane. After 10 minutes of vigorous stirring, the layers were separated and the aqueous portion washed with three 75 ml aliqouts of dichloromethane. The organic portions were combined, dried over magnesium sulfate, and evaporated to obtain 3.6 g of 1-(10-acetoxy-11-bromoundecanyl)-3,7-dimethylxanthine. Without further purification, the bromoacetate was taken up in 25 ml of methanol and treated with a solution of sodium methoxide (prepared from 0.28 g, 12.2 mmol sodium, and 25 ml methanol). After 30 minutes, most of the solvent was removed under reduced pressure and the residue was extracted with three 75 ml aliquots of dichloromethane. The organic portions were combined, dried over magnesium sulfate and concentrated under reduced pressure to give an off-white solid. Subsequent purification by column chromatography over silica gel using a dichloromethane/(3%) methanol eluant produced 2.0 g of 1-(10,11-oxidoundecanyl)-3,7-dimethylxanthine (57.5% yield).

312 mg of sodium hydride (13 mmol) were added to a solution of 10 ml of octanol in 20 ml of toluene. After the solution ceased bubbling, 2.5 g 1-(10,11-oxidoundecyl)-3,7-dimethylxanthine (7.2 mmol) was added and the mixture was stirred for 3 hours at 60-70 °C. After cooling, the mixture was added to a saturated aqueous solution of 15 ml of ammonium chloride and 10 ml of water and extracted with three 50 ml aliquots of dichloromethane. The combined organic layers were washed with saturated aqueous salt solution and dried over sodium sulfate. Evaporation of the solvents under vacuum gave a solid residue which was purified by chromatography (neutral activity II alumina/dichloromethane) to obtain 1.34 g of 1-(11-octyloxy-10-hydroxyundecyl)-3,7-dimethylxanthine (49% yield).

0.18 ml of dimethylsulfoxide (2.5 mmol) was added slowly by syringe to a stirring solution of 0.11 ml of oxalyl chloride (1.3 mmol) in 5 ml of dichloromethane -78 °C. After 2 minutes, a solution of 0.5 g of 1-(11-octyloxy-10-hydroxyundecyl)-3,7-dimethylxanthine (1.0 mmol), prepared above, in 15 ml of dichloromethane was added dropwise over 5 minutes. Stirring was

continued at -78 °C for 45 minutes. 0.72 ml of triethylamine (5.2 mmol) was added and the cold bath removed. After 20 minutes, 25 ml of water were added and the mixture was extracted with three 50 ml aliquots of dichloromethane. The combined organic extracts were washed with 20 ml of saturated aqueous sodium bicarbonate and 20 ml of saturated aqueous salt solution, and then dried over sodium sulfate. The solvents were evaporated under vacuum, leaving a thick oil residue which was purified by chromatography using silica and a dichloromethane-5% methanol eluant to obtain 205 mg of compound no. 2594 (43% yield).

Example 6

A solution of 1.0 g of 1-methylthymine (7.1 mmol), available from Aldrich, and 171 mg of sodium hydride (7.1 mmol) in 20 ml of dimethylsulfoxide was stirred at ambient temperature. After 45 minutes, 1.30 g of 6-bromo-2-hexanone (7.1 mmol) was added and the reaction mixture was stirred for 72 hours. The reaction was poured into 50 ml water and extracted with four 50 ml aliquots of dichloromethane. The organic layers were combined and washed with two 30 ml aliquots of saturated aqueous salt solution and dried over magnesium sulfate. Evaporating the solvent left a brownish solid, which upon recrystallization in dichloromethane/petroleum ether yielded 960 mg of a white solid, compound no. 1909 (56% yield).

Example 7

This example is a synthesis for inventive compound no. 2521. 11.9 g of theobromine (66 mmol), available from Sigma, were added to a mixture of 10.7 g of bromohexene (66 mmol), available from Aldrich, and 1.58 g of sodium hydride (66 mmol) in 100 ml of dimethylsulfoxide and the resulting reaction mixture stirred for 43 hours. The solution was treated with 200 ml of water and then extracted with three 80 ml aliquots of dichloromethane. The combined extracts were washed with three 100 ml aliquots of water, dried over magnesium sulfate, and then the solvent was evaporated under vacuum, leaving 17 g of white powder, 1-(5-hexenyl)-3,7-dimethylxanthine (65 mmol, 98% yield).

Six drops of 2.5% osmium tetraoxide in *t*-butanol were added to a mixture of 1.07 g of 1-(5-hexenyl)-3,7-dimethylxanthine (4.1 mmol) and 1.44 g of *N*-methylmorpholine-*N*-oxide (12.3 mmol) in 20 ml of water and 10 ml of acetone. After stirring a resulting reaction mixture for 48 hours, the mixture was treated with 20 ml of 20% aqueous sodium dithionite solution. After 2 minutes, the mixture was extracted with three 30 ml aliquots of 25% ethanol/dichloromethane solution. The combined extracts were dried over magnesium sulfate. Evaporating the solvent under vacuum, left 750 mg of white powder, 1-(5,6-dihydroxyhexyl)-3,7-dimethylxanthine (2.53 mmol, 62% yield).

3.4 ml of 30% hydrogen bromide-acetic acid were added over 30 seconds to 1.0 g of 1-(5,6-dihydroxyhexyl)-3,7-dimethylxanthine (3.38mmol), prepared above, and then the resulting solution was stirred 2.5 hours until all of the solid had dissolved. The solution was poured carefully over a mixture of 12 g of sodium bicarbonate and 50 ml of ice water (50 ml). After the carbon dioxide evolution had subsided, the mixture was extracted with three 25 ml aliquots of dichloromethane. The combined extracts were dried over magnesium sulfate and the solvent was evaporated under vacuum, resulting in 1.3 g of a viscous oil, 1-(5-acetoxy-6-bromohexyl)-3,7-dimethylxanthine (3.24 mmol, 96% yield), The viscous oil residue was dissolved in 5 ml of methanol to form a residue solution. 3.9 ml of a 1*M* sodium methoxide in methanol solution was added to the residue solution over 30 seconds. After stirring for 20 minutes, the solution was treated with 20 ml of water and then extracted with three 15 ml aliquots of dichloromethane. The combined extracts were dried over magnesium sulfate. Evaporating the solvents under vacuum left 900 mg of white crystals, 1-(5,6-oxidohexyl)-3,7-dimethylxanthine (3.24 mmol, 100% yield).

120 mg of a solution of 1-(5,6-oxidohexyl)3,7-dimethylxanthine (0.43 mmol), prepared above, in 10 ml of methanol were refluxed with six drops of sulfuric acid for 4 hours. The resulting reaction mixture was cooled and then 10 ml of saturated sodium bicarbonate solution were added. The mixture was extracted with three 30 ml aliquots of 10% ethanol/dichloromethane and the organic layers were combined and dried over sodium sulfate. The solvents were evaporated, leaving a yellow oil. Purification by chromatography using silica and a 10% ethanol/dichloromethane eluant and by subsequent crystallization from dichloromethane/petroleum ether yielded 44 mg of a white solid, 1-(6-methoxy-5-hydroxyhexyl)-3,7-dimethylxanthine (33% yield).

223 mg of oxalyl chloride (1.8 mmol) were cooled to -78 °C in 8 ml of dichloromethane. 281 mg of dimethylsulfoxide (3.6 mmol) were added dropwise to the cooled solution. After 5 minutes, 500 mg of 1-(6-methoxy-5-hydroxyhexyl)-3,7-dimethylxanthine (1.6 mmol), prepared above, in 6 ml of dichloromethane were added dropwise. Stirring continued for 40 minutes, thereafter. 729 mg of triethylamine (7.2 mmol) were added to the reaction mixture. The reaction mixture was allowed to warm to ambient temperature. The reaction mixture was poured into 20 ml of water and extracted first with three 40 ml aliquots of dichloromethane, and then followed by 30 ml of 20% methanol/dichloromethane. The organic layers were combined and washed with 20 ml of aqueous saturated salt solution and dried over sodium sulfate. Evaporation of the solvent yielded a yellow residue, which upon dissolving and cooling in dichloromethane and hexane resulted in a yellowish solid. Purification by chromatography of the resulting solid using silica and a dichloromethane/5% ethanol eluant produced 202 g of compound no. 2521 (41% yield).

Example 8

This example is a synthesis for inventive compound no. 3525. 1-(10,11-Dihydroxyundecanyl)-3,7-dimethylxanthine (3.6 g, 10 mmol), prepared in Example 5 above, was stirred with a 30 % solution (6.2 ml) of hydrogen bromide (8.4 g, 31.1 mmol) in acetic acid for 90 minutes. The mixture was then added to a flask containing aqueous sodium bicarbonate solution (100 ml) and dichloromethane (75 ml). After 10 minutes of vigorous stirring, the layers were separated and the aqueous portion washed with three aliquots of dichloromethane (75 ml each). The organic portions were combined and dried over magnesium sulfate. The solvents were evaporated, yielding 3.6 g of 1-(10-acetoxy-11-bromoundecanyl)-3,7-dimethylxanthine. Without further purification, this resulting bromoacetate was taken up in methanol (25 ml) and treated with a solution of sodium methoxide (prepared from 0.28 g, 12.2 mmol sodium, and 25 ml methanol). After 30 minutes, most of the solvent was removed under reduced pressure and a residue extracted with three aliquots of dichloromethane (75 ml each). The organic portions were combined, dried over magnesium sulfate and concentrated under reduced pressure, resulting in an off-white solid. Purification of the off-white solid by column chromatography over silica gel using a dichloromethane/(3%) methanol eluant produced 2.0 g of 1-(10,11-oxidoundecanyl)-3,7-dimethylxanthine (57.5% yield).

A 0.31 M solution (8 ml) of nonylmagnesium bromide (2.50 mmol) in tetrahydrofuran was added to a suspension of copper iodide (0.095 g, 0.50 mmol) in tetrahydrofuran (16 ml) at -40 °C and the mixture stirred for 40 minutes at -40 °C. A solution (10 ml) of 1-(10,11-oxidoundecanyl)-3,7-dimethylxanthine (0.70 g, 2.00 mmol), produced above, in dry tetrahydrofuran was added to the mixture and the combined mixture stirred at -40 ° C for 90 minutes. Hydrochloric acid (10 ml of 1.0 M) was added to the mixture and a product extracted with two aliquots of dichloromethane (30 ml each). The combined organic phase was dried over magnesium sulfate and the solvent was evaporated, leaving a residue, which was subsequently purified by column chromatography using an ethyl acetate eluant, producing a white solid, 1-(10-hydroxyeicosyl)-3,7-dimethylxanthine (0.25 g, 27% yield).

Dimethylsulfoxide (0.063 ml, 0.82 mmol) and a solution (2 ml) of 1-(10-hydroxyeicosyl)-3,7-dimethylxanthine (0.135 g, 0.28 mmol), prepared above, in dichloromethane were added over a period of 5 minutes to a solution (3 ml) of oxalyl chloride (0.036 ml, 0.41 mmol) in dichloromethane at -78 °C. The resulting reaction mixture was stirred at -78 °C for 30 minutes, and after addition of triethylamine (0.23 ml, 1.64 mmol), was allowed to warm over 30 minutes to 25 °C. Hydrochloric acid (1.0 M, 10 ml) and dichloromethane (5 ml) were added to the warmed mixture, the organic phase separated and the aqueous phase washed with dichloromethane (10 ml). The organic portions were combined, dried over

magnesium sulfate, and the solvent evaporated, leaving a slightly yellow solid. Purification by chromatography using silica and an ethyl acetate eluant, produced 0.110 g of a white solid, compound no. 3525 (85% yield).

Example 9

This example shows an inhibitive effect of inventive compounds nos. 1595 and 2510 on murine thymocyte proliferation stimulated by Concanavalin A (ConA) and interleukin-2 (IL-2). This assay is an *in vitro*, predictive model of a compound's therapeutic potential in treating or preventing autoimmune, immune or inflammtory diseases. Procedurally, thymuses were obtained from normal, female Balb/C mice. The thymuses were dissociated and plated into 96-well plates at a density of 2×10^5 cells/well. ConA (0.25 mg/ml) and IL-2 (12.5 ng/ml) were added to the wells. Drug was added at various doses two hours prior to activation with ConA and IL-2. The cells were incubated for 4 days at 37 °C. On day 4, the cells were pulsed with tritiated thymidine and allowed to incubate for an additional 4 hours. Harvested cells were analyzed for incorporated tritiated thymidine, determined using a liquid scintillation counter. Dose response curves were prepared from the assay results and used to calculate an IC50 value for each compound tested.

Dose response curves figures 1 and 2 illustrate the inhibitive effects of compounds nos. 1595 and 2510, respectively, on proliferation of thymocytes. Background counts, without addition of representative inventive compounds were slightly above 200 cpm. Figure 1 illustrates an ability of compound no. 1595, representative of aldehyde-substituted inventive compounds, to inhibit ConA/IL-2 stimulated proliferation at compound concentrations less than 2.5 μM. Similarly, Figure 2 shows that compound no. 2510, representative of ketone-substituted inventive compounds, also inhibits thymocyte proliferation, most noticeably at compound concentrations less than 20 μM. These concentrations are within known *in vivo* concentrations useful in treating disease. Experimentally calculated IC50 values for the inventive compounds nos. 1595 and 2510 in these assays are 8.7 nM and 4.3 μM, respectively.

Example 10

This example illustrates an ability of inventive compounds nos. 1516, 1526, 1810 and 2594 to inhibit proliferation of peripheral blood mononuclear cells (PBMC) in response to allogeneic stimulation. This *in vitro* mixed MLR assay is useful in assessing activity of an inventive compound. Procedurally, PBMC were obtained by drawing whole blood from healthy volunteers in a heparinized container, the whole blood samples diluted with an equal volume of hanks balanced salt solution (HBSS).

This mixture was layered on a sucrose density gradient, such as a Ficoll-Hypaque® gradient (specific gravity 1.08), and centrifuged (1000 x g) for 25 minutes at no warmer than room temperature. PBMC were obtained from a band at a plasma-Ficoll interface, separated and washed at least twice in a saline solution, such as HBSS. Contaminating red cells were lysed, for example, by ACK lysis for 10 minutes at 37 °C, and the PBMC were washed twice in HBSS. The pellet of purified PBMC was resuspended in complete medium, such as RPMI 1640 plus 20% human inactivated serum.

Proliferative response of PBMC to allogeneic stimulation was determined in a two-way MLR performed in a 96-well microtiter plate. Approximately 10^5 test-purified PBMC in 200 µl complete medium were co-cultured with approximately 10^5 autologous (control culture) or allogeneic (stimulated culture) PBMC. Allogeneic cells were from HLA disparate individuals. Varying doses of compounds nos. 1516, 1526, 1810 and 2594 were added simultaneously upon addition of cells to the microtiter plate. The cultures were incubated for 6 days at 37 °C in a 5% CO_2 atmosphere, after which time, tritiated thymidine was added (for example, 1 µCi/well of 40 to 60 Ci/mmole) and proliferative inhibition was assessed by determining amount of tritiated thymidine taken up, using liquid scintillation counting.

Figures 3, 4, 5 and 6 are plotted graphs of compound concentrations (µM) versus incorporated thymidine (cpm), for compounds nos. 1516, 1526, 1810 and 2594, respectively. Figure 3 illustrates a most pronounced inhibition of PBMC proliferation. At concentrations less than 50 µM, compound no. 1516 noticeably inhibited incorporation of thymidine. Similarly, figures 4 and 6 illustrate inhibitive characteristics of inventive compounds nos. 1526 and 2594, respectively, in this MLR assay at compound concentrations less than 250 µM. Figure 5 illustrates some inhibition, although to a lesser degree than other compounds tested in this assay.

Example 11

This example illustrates a method for assessing an effect on normal cells of the inventive compounds, considered potential cancer therapies. This assay has been used clinically to evaluate recovery of patients' marrow following chemotherapy or radiation. In this specific example, inventive compound no. 1595 is less deleterious to normal cells in comparison to a known chemotherapeutic compound.

Mouse bone marrow cells are useful in this assay because they produce colonies, which can later be counted, in culture. The colonies are called colony forming unit-granulocyte macrophage (CFU-GM) and depend on a source of colony stimulating factor for growth.

Mouse spleen conditioned-medium, at a concentration of 2%, was used in this assay. The medium and semi-solid culture mix were procurred from Stem Cell Technologies in Vancouver BC. In animal studies performed in a related cytoreductive treatment analysis, no

CFU-GM were detectable in mouse femoral marrow during immediate days following 5-fluorouracil or thiotepa treatment.

Cells were cultured with a known chemotherapeutic agent or inventive compound no. 1595, to comparatively evaluate an effect on normal cells of inventive compounds identified as potential cancer therapies. Procedurally, cells were incubated for 8 hours with various concentrations of compound no. 1595, a representative ketone-substituted compound of the invention having cancer therapy potential, or vinblastin, a known chemotherapeutic agent. After 8 hours, the incubated cells were washed thoroughly and a consistant number were subsequently plated to obtain CFU-GM. Colonies were permitted to grow for 7 days at 37 °C, in 5% CO_2. After 7 days of growth, colony growths were counted microscopically. Data obtained in this assay is plotted in figure 7. Figure 7 compares the colonies counted for cells incubated with compound no. 1595 and a comparative chemotherapeutic agent. At concentrations ranging from 5 to 30 μM, more colonies existed for cells incubated with the inventive potential cancer compound than for cells incubated with vinblastin, suggesting less deleterious effects in cancer therapies using the inventive compounds in comparison with conventional treatments.

Example 12

This example illustrates an ability of inventive compounds nos. 2591 and 2594 to inhibit adherence of specific cells to (HUVEC) stimulated with either IL-1β or TNFα. Abnormal cell-surface receptor signaling has an important role in aggravating or promoting chemotaxis in immune, inflammatory, cancers and other diseases. This adhesion assay is useful in showing an ability of an inventive compound to inhibit adhesion of a specific cells induced by the signaling phenomenon disclosed herein, and thus in predicting therapeutic potential of the inventive compounds.

Two days prior to conducting the assay procedure, HUVEC were plated at 4000 cells/well. After two days, HUVEC were stimulated overnight with IL-1β (20 ng/ml and 10 ng/ml) or TNFα (20ng/ml). Cells, chosen from among: Jurkat (a human, acute, leukemia T cell line); white blood cell neutrophils; and THP-1 (a human acute monocytic leukemia cell line) were prestained with 2,7-bis-(2-carboxyethyl)-5(and-6)carboxyflourescein,acetoxymethyl ester (BCECF--a compound that when cleaved by esterases, yields a flourescent product, thus providing a measure of cell number) and added at 250,000 per well in RPMI containing 1% fetal calf serum. Proper controls were maintained and simultaneously analyzed, both with and without inventive compound and IL-1β or TNFα. The cells are allowed to adhere for 20 minutes at 37 °C, after which time, the plate is inverted and spun at 800 rpm. The wells are washed once with PBS and resuspended in 100 μl PBS prior to reading fluorescence on a

Millipore fluorescence plate reader. Data is recorded as percent adherence (Jurkat, Neutrophil or THP-1 cells to HUVEC) at selected concentrations of compounds nos. 2591 or 2594.

Figures 8, 9, 10, 11 and 12 report plotted results of data obtained in this assay. Figure 8 represents experimentally determined effects of compound no. 2591 on percent adherence of Jurkat cells to TNFα-stimulated HUVEC. Figure 9 corresponds to data obtained for compound no. 2591 on adherence of THP-1 cells to TNFα-stimulated HUVEC. Figure 10 illustrates an ability of compound no. 2594 to inhibit adherence of THP-1 cells to TNFα-stimulated HUVEC and figures 11 and 12 represent an ability of compound no. 2591 to inhibit THP-1 and neutrophil cell adherence to IL-1β stimulated HUVEC, respectively. In all assay conditions, the representative, inventive compounds inhibit adherence at compound concentrations less than 10 μM, suggesting that the compounds tested, as representatives of all inventive compounds, possess therapeutic potential in diseases exhibiting characteristics of this signaling phenomenon.

Example 13

This example illustrates that inventive compound no. 2591 inhibits surface expression of specific surface receptors. Specifically, compound activity was measured by determining levels of intracelluar adhesion molecule.(ICAM) or vascular cell adhesion molecule (VCAM) in stimulated normal cells. Early passage HUVEC--obtained from commercial suppliers such as Cell Systems, Inc. or Clonetics--were cultured in media containing 10% fetal bovine serum, and stimulated with TNFα or IL-1β (20 ng/ml). The cells were plated into wells of a microtiter plate (*e.g.*, 5 x 10^4 per well) and allowed to incubate at 37 °C for 20 hours. The resting cells were removed, washed in media with a phosphate-buffered saline plus 0.1% bovine serum albumin and 0.01% sodium azide and labeled on ice with a monoclonal antibody ("first antibody") recognizing either ICAM or VCAM. After 60 minutes on ice, the cells were washed with cold wash media and incubated with an antibody that recognizes the first antibody (1 μg of goat anti-mouse IgG conjugated with phycoerythrin recognizing the monoclonal antibody for VCAM, and goat anti-mouse antibody conjugated to fluorescein-isothiocyanate (FITC) recognizing the monoclonal antibody for ICAM). After 30 minutes on ice, the cells were washed twice and analyzed on a flow cytometer (Coulter Elite®) at appropriate emission and excitation wavelengths to determine mean fluorescence intensity, correlating to ICAM or VCAM expression.

Figures 13, 14, 15 and 16 report % surface expression of ICAM or VCAM as a function of mean fluorescence intensity versus concentration of inventive compound. Figures 13 and 14 illustrate that inventive compound no. 2591 inhibits surface expression of VCAM in normal HUVEC stimulated with 20 ng/ml of TNFα and IL-1β, respectively. Figures 15 and 16

report that inventive compound no. 2591 inhibits surface expression of ICAM in normal HUVEC stimulated with 20 ng/ml of TNFα and IL-1β, respectively. The data in these figures suggests that the tested compound, representative of compounds of the invention, at concentrations less than 10 μM interfere with ICAM and VCAM receptor signaling mechanisms and thus would be useful in treating or preventing diseases characterized by these or a similar mechanism.

Example 14

This example illustrates inhibitive effects of the inventive compounds on Balb/3T3 cell proliferation in response to platelet derived growth factor (PDGF) stimulation.

Disregulated PDGF-proliferative response has been linked to a variety of diseases, including, *e.g.*, restenosis, atherosclerosis, fibrosis, and tumor cell angiogenesis. Balb/3T3 cells respond vigorously to PDGF stimulation, and are useful *in vitro* models for further study of PDGF-induced proliferation. In an assay useful in determining whether a compound would be useful in treating diseases characterized by this or similar disregulated proliferative responses, research indicates that the inventive compounds inhibit PDGF-induced proliferation of Balb/3T3 cells.

Balb/3T3 cells were plated in low serum-containing medium for 24 hours prior to stimulation with various concentrations of inventive compound no. 3510. PDGF was added at varying concentrations along with tritiated thymidine. The cells were allowed to incubate for one day, following addition of PDGF and thymidine. 24 hours later, the cells were harvested and counted by liquid scintillation counting. Figure 17 reports data obtained in this proliferation assay. The results illustrate that compound no. 3510 inhibits proliferation of Balb/3T3 cells stimulated by PDGF at concentrations less than 30 μM, indicating that the inventive compounds are candidates for treating or preventing restenosis, atherosclerosis, fibrosis, tumor cell angiogenesis and other similar diseases.

In conjunction with the Balb/3t3 proliferation assay, a related viability assay was conducted to assess the cytotoxicity of compounds which inhibit proliferation in this system. The assay protocol was identical to that performed above except that tritiated thymidine was not added after the 24 hour incubation with PDGF. Subsequent to incubation, a 10 μM solution of BCECF was added and the cells incubated for 30 minutes at 37 °C. Following this incubation, BCECF was replaced with PBS and the plate read for flourescence in a Millipore cytofluorometer. Data obtained was plotted as a percent of control versus concentration of inventive compound tested. Figure 18 represents the results of this viability assay. The compound tested, representative of compounds of the invention, was not cytotoxic to any cells

(as compared with a control value of 100 %) at concentrations shown in figure 17 to inhibit proliferation.

Example 15

This example illustrates the inventive compounds nos. 1595, 2510, 2524 and 3510 inhibit LPS-induced TNF release in whole human blood.

Whole blood was collected from a healthy human donor into vacutainer tubes containing ACD citrate as anti-coagulant. The compounds tested were diluted in RPMI medium and 5µl of the dilute concentrations placed in tubes containing 225 µl of whole blood. The tubes were mixed and incubated for no more than 1hour at 37 °C. LPS Salmonella abortus equi (commercially available from Sigma) is diluted in RPMI and the dilute samples added to the whole blood/compound samples at 20 µl per tube (10ng/ml final concentration). The tubes are again mixed and incubated for an additional 4-6 hours at 37 °C. Activity is stopped by adding 750 µl of RPMI to each tube, centrifuging and removing the cells. Supernatants are collected and stored overnight at 4 °C. The supernatant samples are assayed for TNF release using immunoassay kits (available commercially from Biosource International, Camarillo, CA).

Data collected and reported was plotted as percent inhibition (of a control at 100%) versus concentration of compound tested. A compound showing 0% is not inhibiting LPS-induced TNF release, while a compound showing 40% is inhibiting 40% of the maximum, control LPS response. Figure 19 reports these results for compounds nos. 1595, 2510, 2524 and 3510. Each compound tested inhibited TNF release up to 60% (compound no. 1595) at concentrations less than 50 µM and between 20 and 35% (compounds nos. 1595, 2510 and 3510) at 10 µM. These data indicate the inventive compounds potential therapeutic agents for treating or preventing diseases mediated by inhibiting or preventing TNF secretion.

Comparative Example 1

This example illustrates a comparative analysis of inventive compound no: 1516 and comparative compounds 1505, 1-(5-oxohexyl)-3-methylxanthine (M6), and 1537, 1-(5-oxohexyl)-3,7-dimethylxanthine (PTX). In the murine thymocyte assay of example 9, dose response curves were prepared for each of the comparative compounds and inventive compound no. 1516. Figure 20a reports results obtained in this assay for the comparative compounds. As shown in from these data, the comparative compounds have experimentally determined IC50 values of 59 and 100 µM (1505 and 1537, respectively), with no marked increase in inhibition of thymocyte proliferation at higher concentrations. In sharp contrast, inventive compound no. 1516, a ketone-containing compound, exhibited a five-fold decrease in IC50 value (12 µM) from those reported for comparative compounds. Results obtained from a murine thymocyte assay

with inventive compound no. 1516 are shown in figure 20b. In view of the results obtained with comparative compounds, the superior inhibition of the inventive compound in this an other predictive assays would not have been expected.

What is claimed is:

1. A therapeutic compound, including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof, having the formula:

$$\text{CORE MOIETY} - (R)_j$$

wherein:

j is an integer from one to three;

the core moiety is a heterocyclic moiety having from one to three, five- to six-membered ring structures; and

R may be selected from the group consisting of hydrogen, halogen, hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups, and formula I:

$$-(CH_2)_n-C-(R_1)_p \quad \text{I}$$

at least one R having formula I, wherein:

n is an integer from three to twenty;

p is two or three;

R_1 is selected from the group consisting of hydrogen, halogen, hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group, =O, $-(CH_2)_s-C(R_2)_t$, s being zero or an integer from one to ten, t being two or three, R_2 being hydrogen, halogen, hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group, or =O;

at least one R_1 or one R_2 is =O; and

a second R_1 or second R_2, bonded to the same, respective -C as the at least one R_1 or one R_2, is other than =O or hydroxide,

with the proviso that when the core moiety is a xanthinyl or xanthinyl derivative, a total number of carbon atoms between the xanthinyl and the =O of R_1 or R_2 is not less than eight.

2. The compound according to claim 1, wherein at least one of $(CH_2)_n$ or $(CH_2)_s$ is substituted by a hydrogen or halogen atom, or a hydroxyl, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group.

3. The compound according to claim 1, wherein n is an integer from eight to eighteen.

4. The compound according to claim 1, wherein n is an integer from ten to eighteen.

5. The compound according to claim 1, wherein p is two; one R_1 of $-C(R_1)_2$ is =O; and the second R_1 is selected from the group consisting of hydrogen, a $C_{(1-10)}$ alkyl, a halo-substituted $C_{(1-10)}$ alkyl, and a substituted or unsubstituted $C_{(1-10)}$ alkoxyl.

6. The compound according to claim 1, wherein the substituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxylalkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic substituent is selected from the group consisting of amido, amino, branched or unbranched $C_{(2-8)}$ alkenyl or $C_{(1-8)}$ alkyl, azido, carbonato, carbonyl, carboxyl, cyano, $C_{(1-8)}$ haloalkyl, isocyano, isothiocyano, phospho, phosphonato, primary, secondary or tertiary hydroxyl, sulfonato, sulfonyl, sulfoxyl, thioamido, thiocarbonato, thionato, thiolnato, thiol, thioureido and ureido.

7. The compound according to claim 6, wherein the $C_{(1-8)}$ haloalkyl substituent is a mono-, di- or tri-substituted haloalkyl.

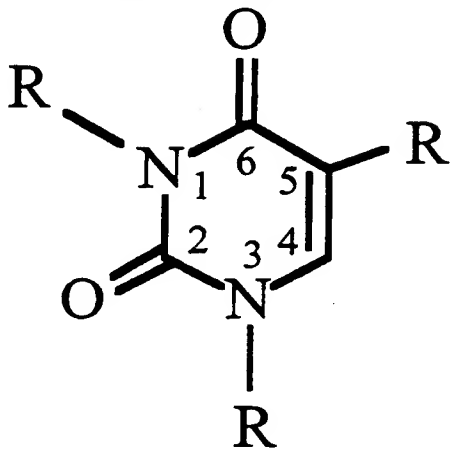
8. The compound according to claim 6, wherein the primary, secondary or tertiary hydroxyl is selected from the group consisting of methanol, butanol, 1-cyclopentanol, ethanol, 2-ethyl-3-methyl-1-propanol, pentanol, propanol and methylcyclohexanol.

9. The compound according to claim 1, wherein the R, R_1 or R_2 cyclic group is selected from the group consisting of anthracenyl, bicyclo[4.4.0]decanyl, bicyclo[2.2.1]heptanyl, bicyclo[3.2.0]heptanyl, bicyclo[4.1.0]heptanyl, bicylo[2.2.1]hexanyl, bicyclo[4.3.0]nonanyl, bicyclo[2.2.2]octanyl, biphenyl, cyclopentadienyl, cyclopentanyl, cyclobutanyl, cyclobutenyl, cycloheptanyl, cyclohexanyl, cyclooctanyl and cyclopropanyl, 1,2-diphenylethanyl, fluorenyl, indenyl, hydroxyphenyl, quinonyl, terphenyl, napthalenyl, phenanthrenyl, terphenyl, toluenyl and xylenyl.

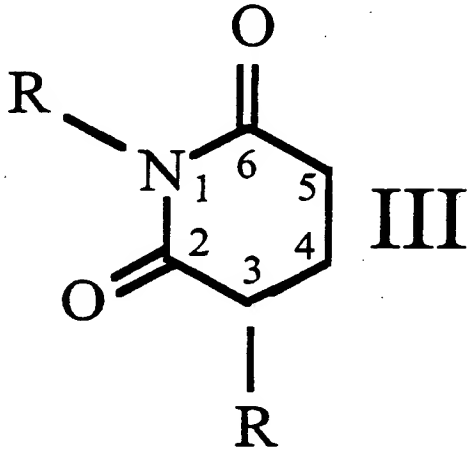
10. The compound according to claim 1, wherein the R, R_1 or R_2 heterocyclic group is selected from the group consisting of azetidinyl, benzofuranyl, benzothiophenyl, carbazolyl, furanyl, glutarimidyl, indolyl, isoquinolinyl, oxazolyl, oxetanyl, oxiranyl, pyrrolidinyl, pyranyl, piperidinyl, pyridinyl, pyrrolyl, quinolinyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydrothiophenyl and thiophenyl.

11. The compound according to claim 1, wherein the heterocyclic core is selected from the group consisting of substituted or unsubstituted barbituryl; benzamidyl; glutarimidyl; homophthalimidyl; hydrophthalimidyl; imidazolyl; indomethacinyl; isocarbostyryl; lumazinyl; N-alkylheterocyclic; N-heterocyclic; pteridinyl; phthalimidyl; piperidinyl; pyridinyl; pyrimidinyl; pyrrolyl; quaternized N-heterocyclic; quinolizinedionyl; quinazolinonyl; quinolinyl; recorsinolyl; succinimidyl; theobrominyl; thyminyl; triazinyl; uric acid; uracyl; vitamins A, E or K and xanthinyl.

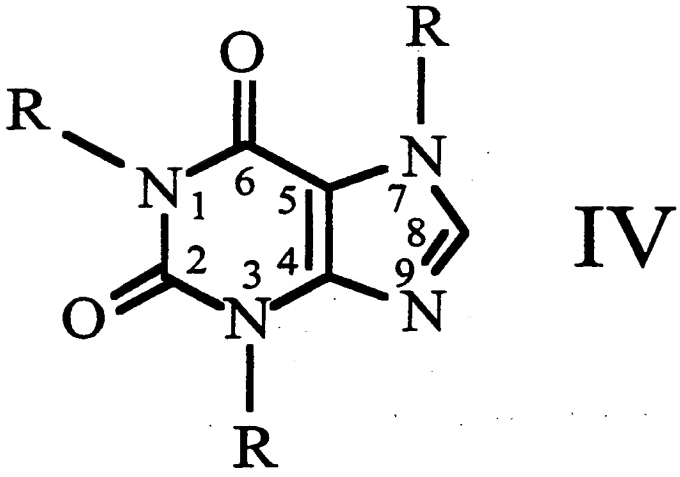
12. The compound according to claim 1, wherein the compound is selected from the group consisting of formulas II, III and IV:



II

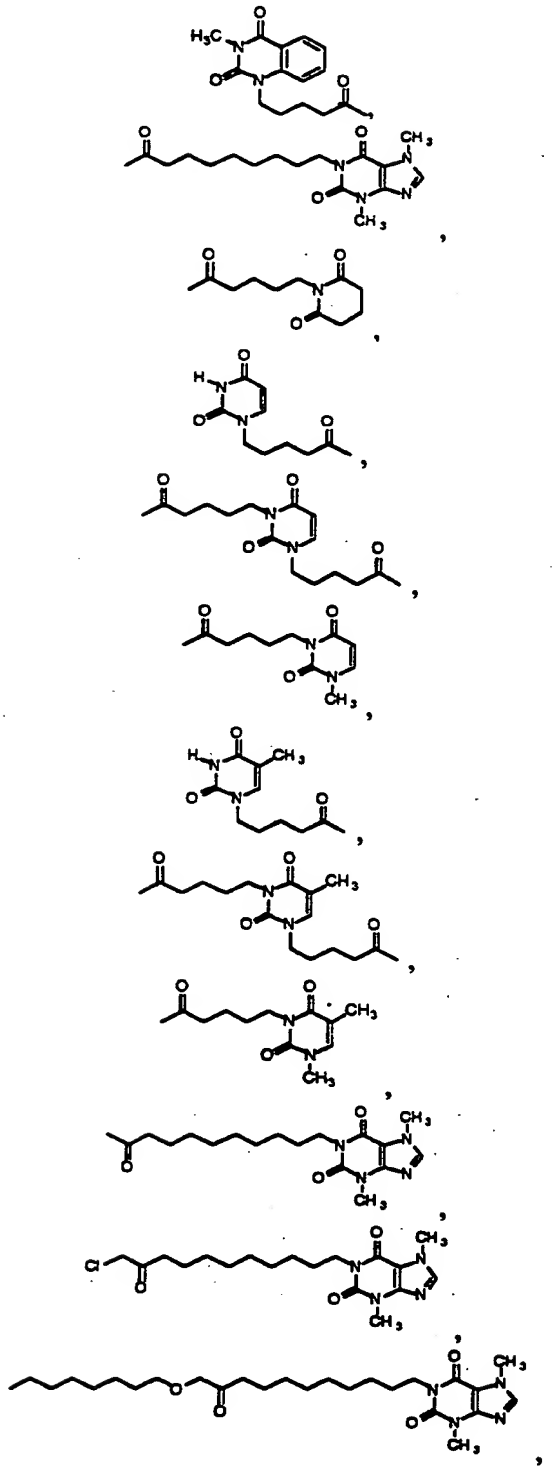


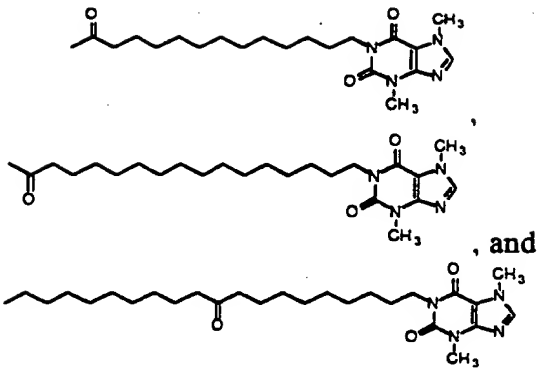
III



IV

13. The compound according to claim 1, wherein the compound is selected from:





14. A pharmaceutical composition comprising a compound of claim 1 and a suitable carrier, diluent or excipient.

15. The pharmaceutical composition of claim 14, wherein the composition is formulated for parenteral, topical or oral administration or for inhalation.

16. A method of synthesizing a compound of claim 1, comprising the steps of:

preparing an intermediate olefinic product;

converting the intermediate olefinic product to a primary or secondary alcohol; and

reacting the primary or secondary alcohol to obtain the compound of claim 1.

17. The method of claim 16, wherein converting the intermediate olefinic product to a primary or secondary alcohol comprises:

reacting the intermediate olefinic product with a hydroborating agent to obtain a borane derivative and converting the borane derivative to a primary or secondary alcohol; or

reacting the intermediate olefinic product converted to obain a corresponding diol, converting the diol to a haloester, and preparing an intermediate epoxide product from the haloester and subsequently reacting the intermediate epoxide product to form the secondary alcohol; or

reacting the intermediate olefinic product with an organic peracid to obtain an intermediate epoxide product directly, and subsequently reacting the intermediate epoxide product to form the secondary alcohol.

18. The method of claim 16, wherein reacting the primary or secondary alcohol comprises converting an hydroxyl functional group to the corresponding aldehyde or ketone functional group by treating the primary or secondary alcohol with a suitable oxidizing agent.

19. A method for treating or preventing acute and chronic inflammatory diseases, AIDS and AIDS related complex, alcoholic hepatitis, allergies due to degranulation of mast cells and basophils, angiogenesis, asthma, atherosclerosis, autoimmune thyroiditis, coronary artery disease, glomerula nephritis, hairloss or baldness, HIV-associated dementia, inflammatory bowel disease, insulin dependent diabetes mellitus, lupus, malignancies, multiple

sclerosis, myelogenous leukemia, organ or hematopoietic in response to cytotoxic therapy, osteoarthritis, osteoporosis, peridontal disease, premature labor secondary to uterine infection, psoriasis, restenosis, rheumatoid arthritis, sleep disorders, septic shock, sepsis syndrome, scleroderma, stroke and transplant rejection in a mammal in need of such treatment, comprising administering an effective amount of a compound the compound of claim 1.

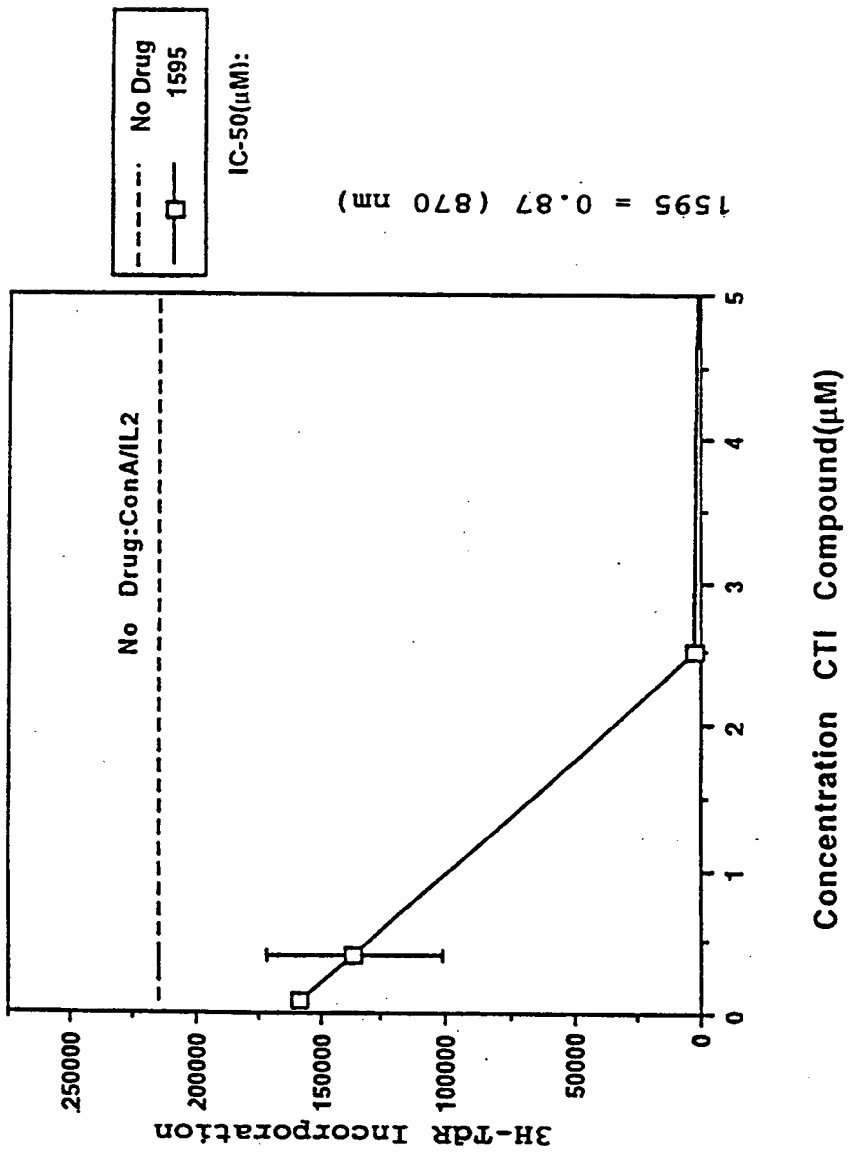


FIGURE 1

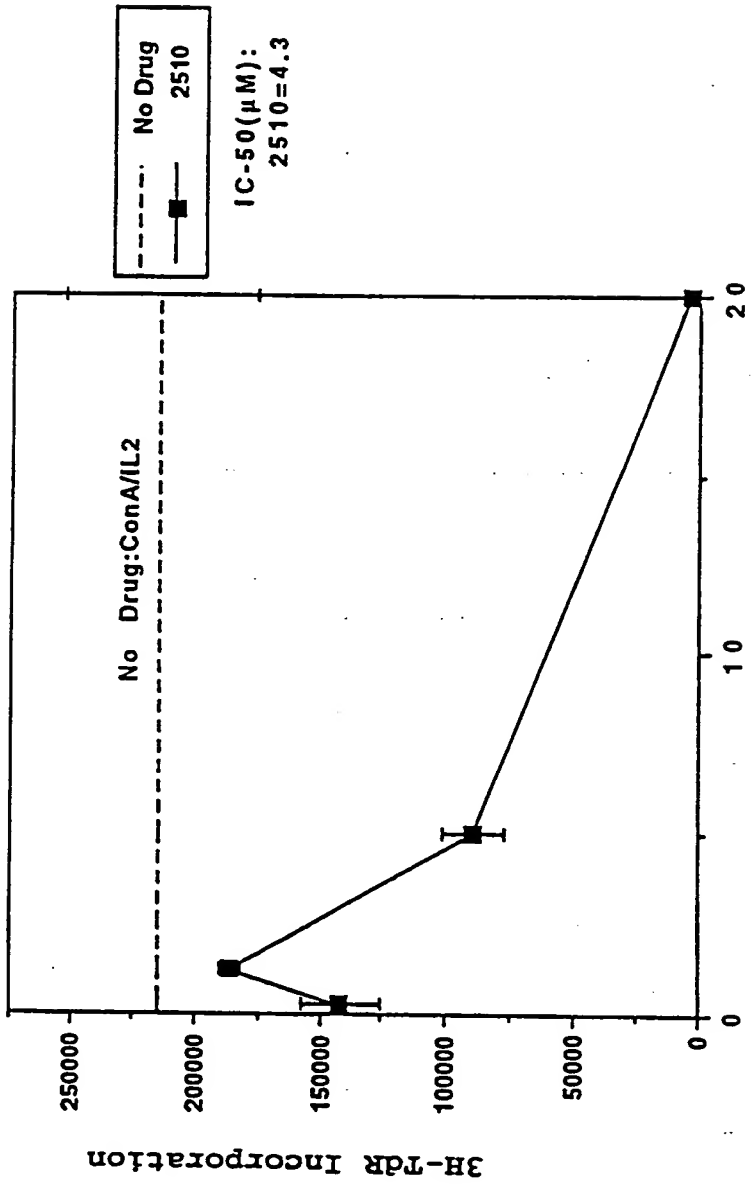
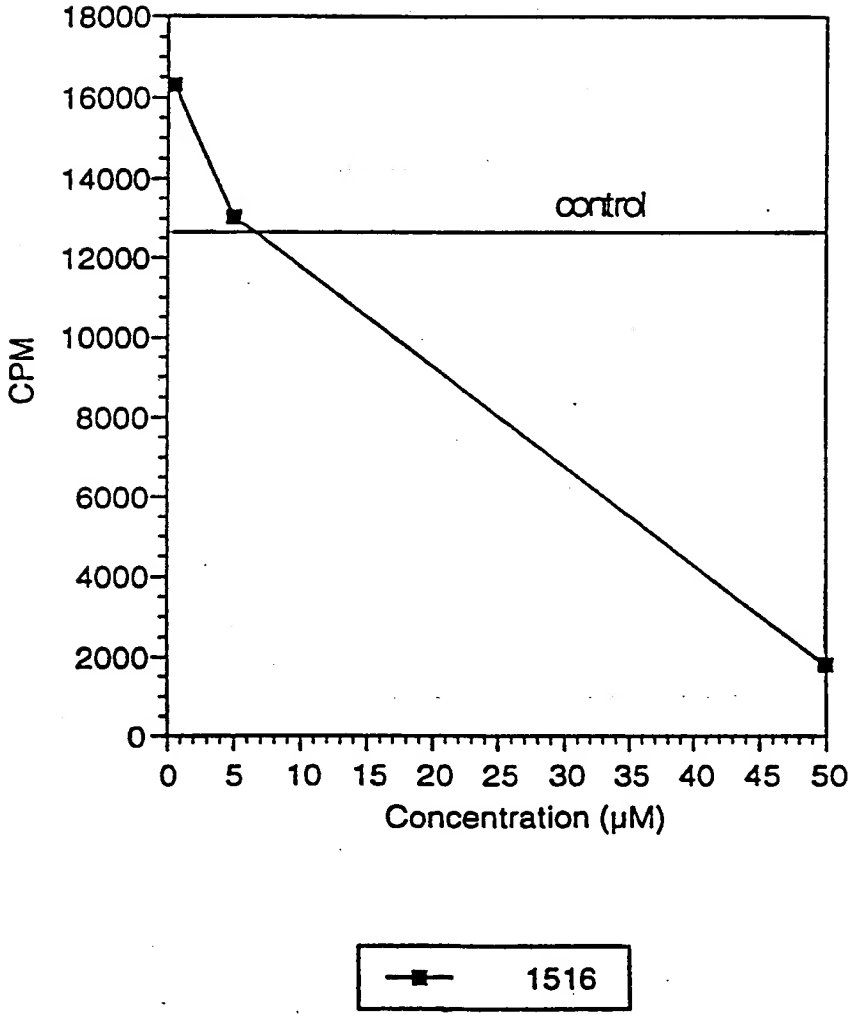
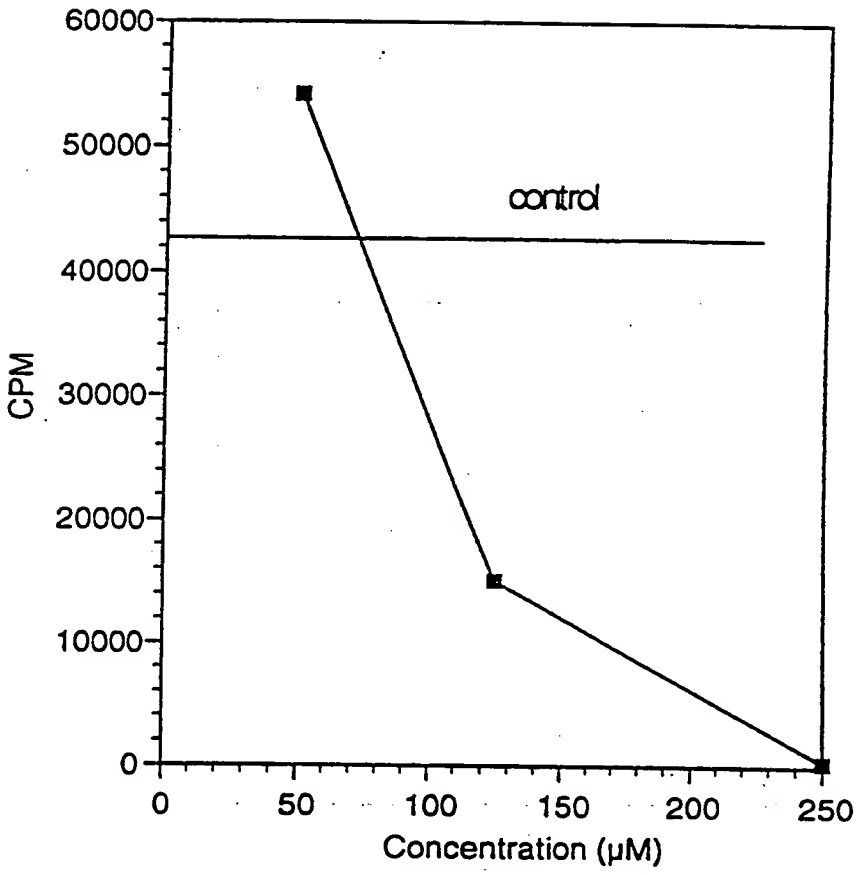


FIGURE 2



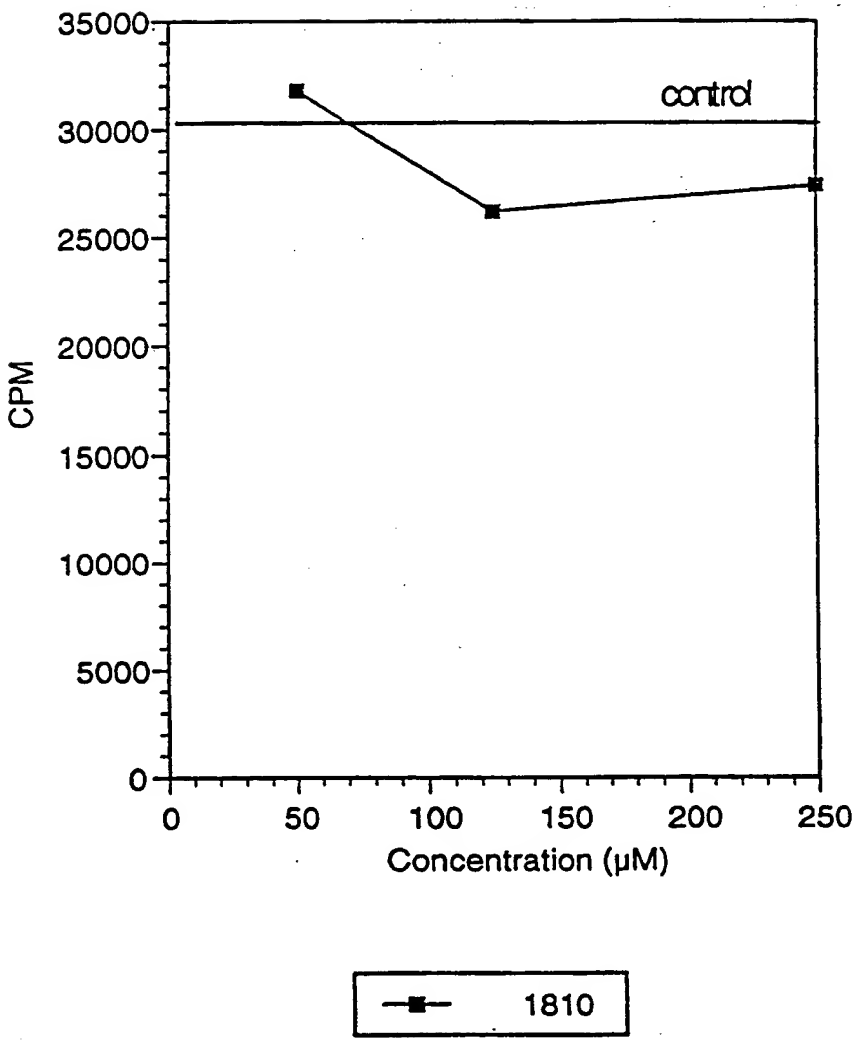
IC-50 27μM

FIGURE 3



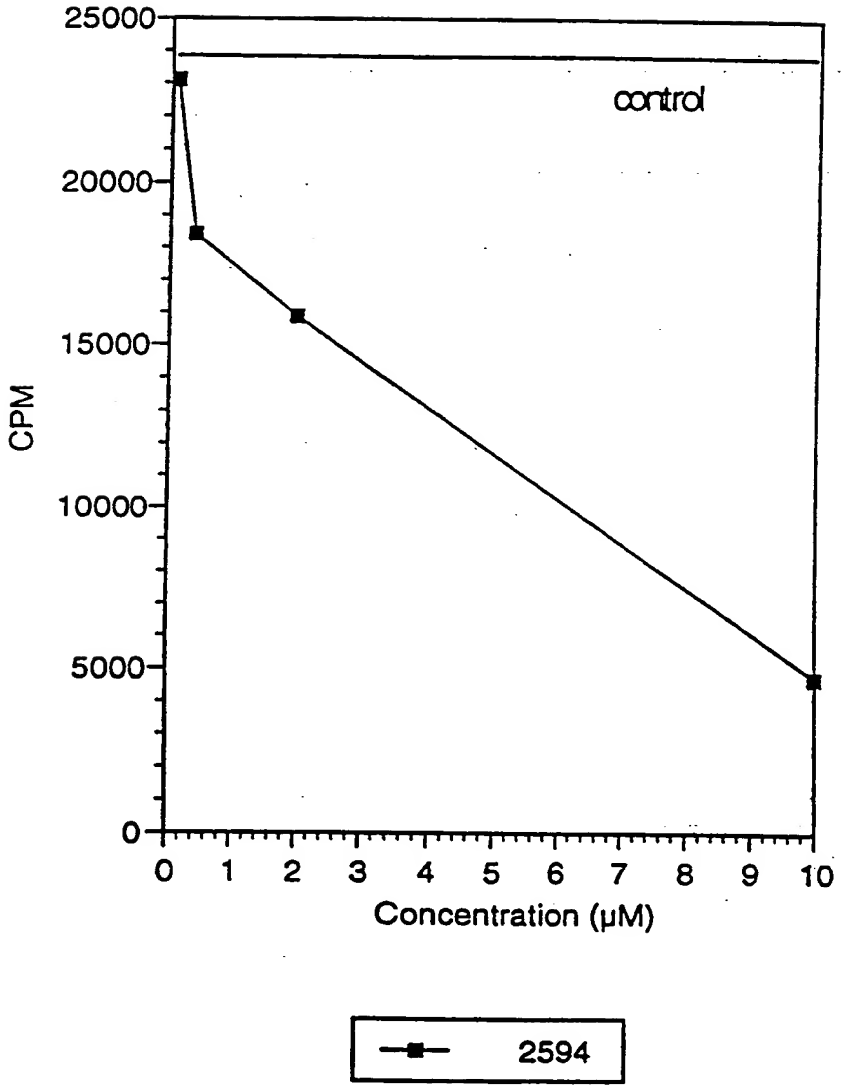
IC-50 115µM

FIGURE 4



IC-50 > 250μM

FIGURE 5



IC-59 4.6μM
FIGURE 6

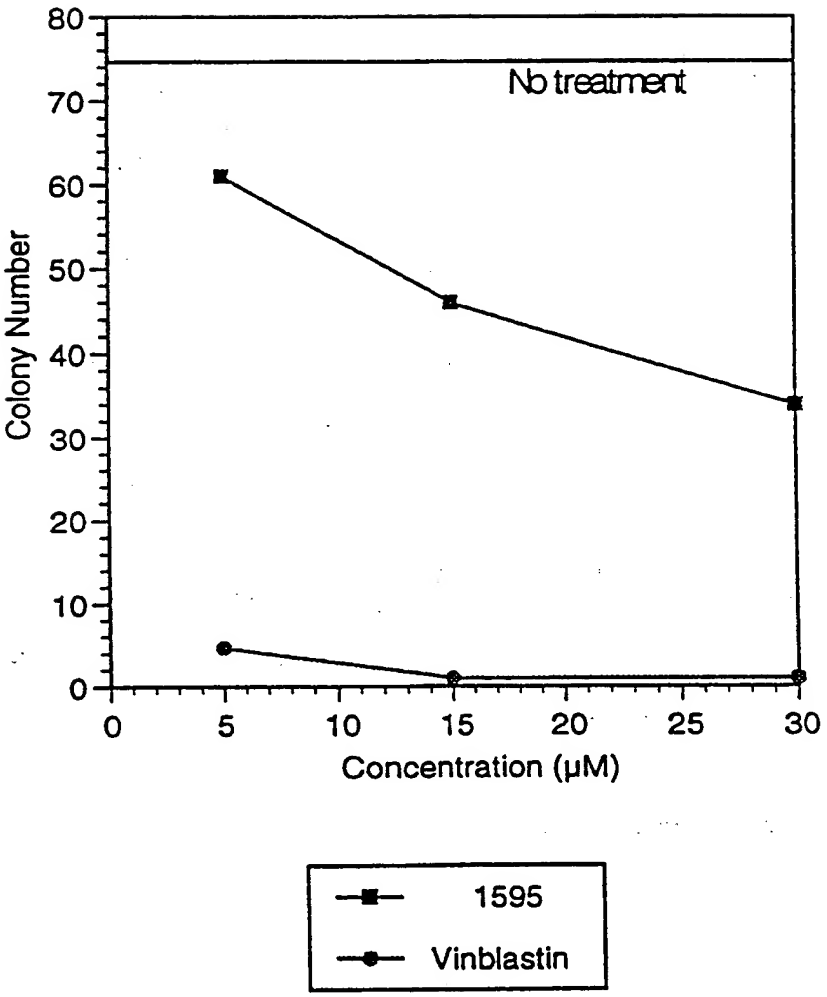


FIGURE 7

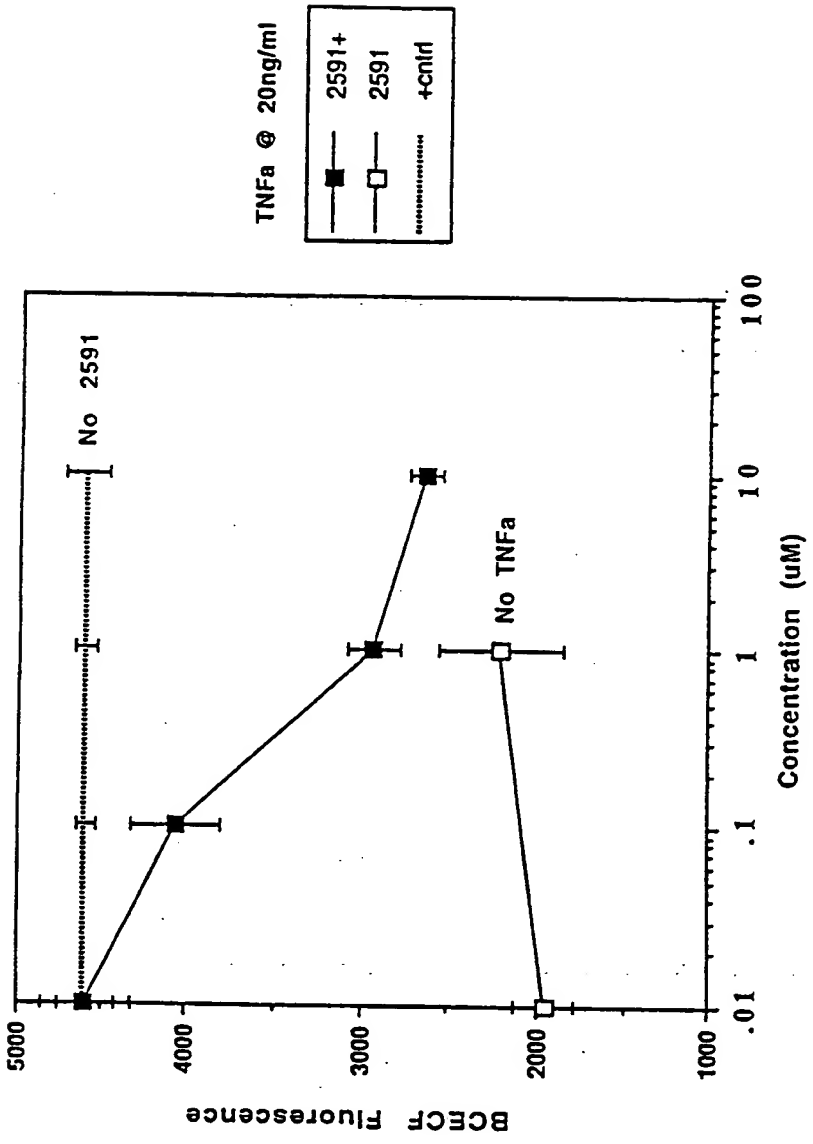

TNFa @ 20ng/ml
2591+
2591
+cntrl
No 2591
No TNFa
BCECF Fluorescence
5000
4000
3000
2000
1000
.01
.1
1
10
100
Concentration (uM)

FIGURE 8

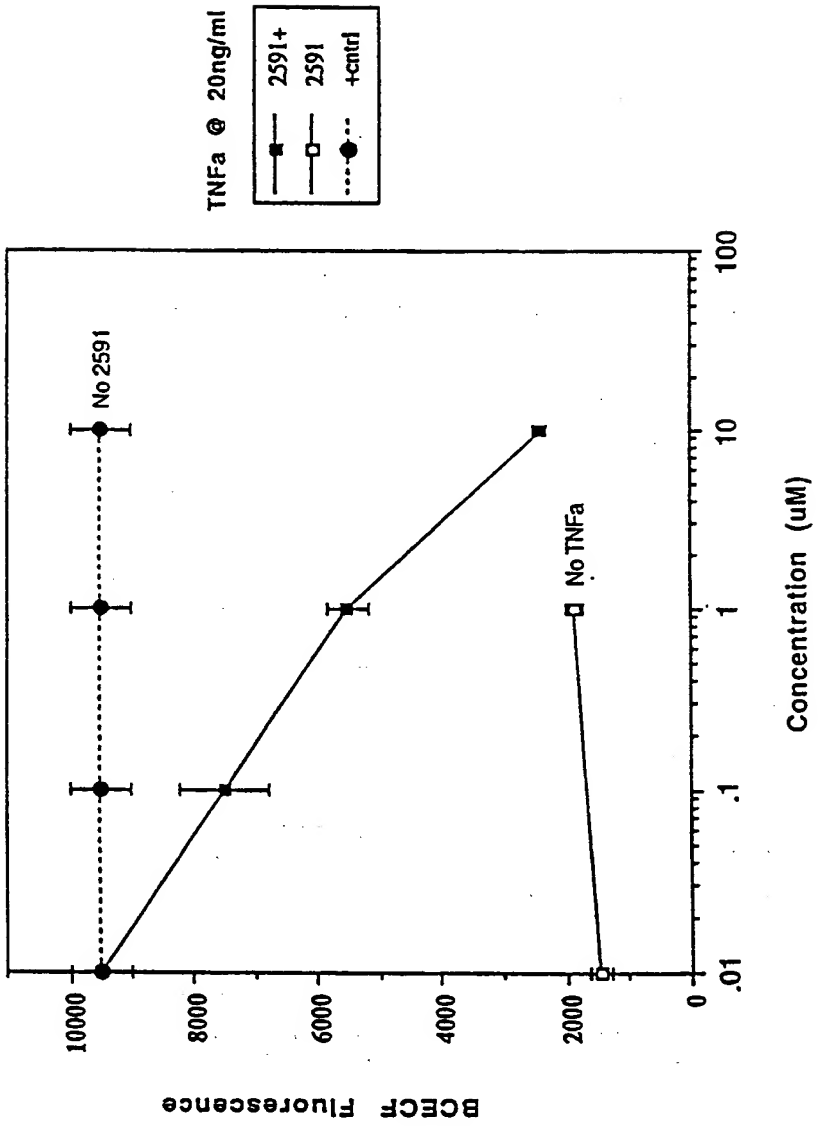


FIGURE 9

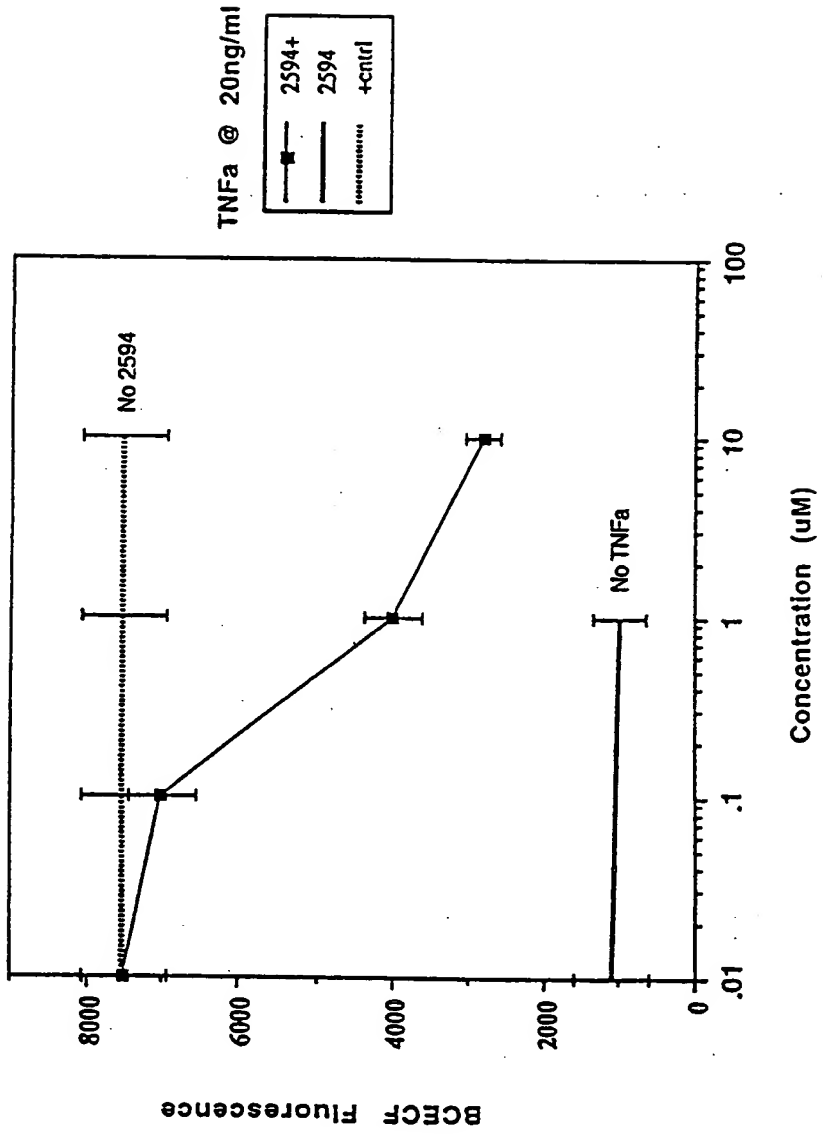


FIGURE 10

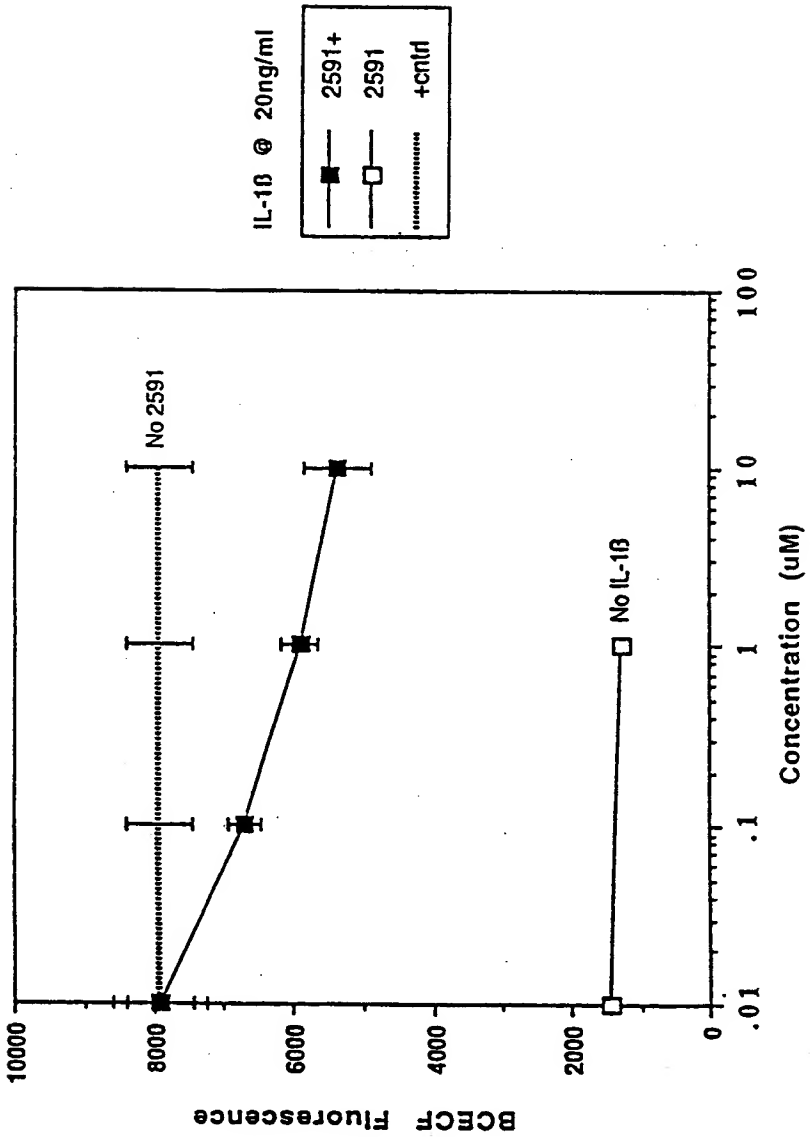


FIGURE 11

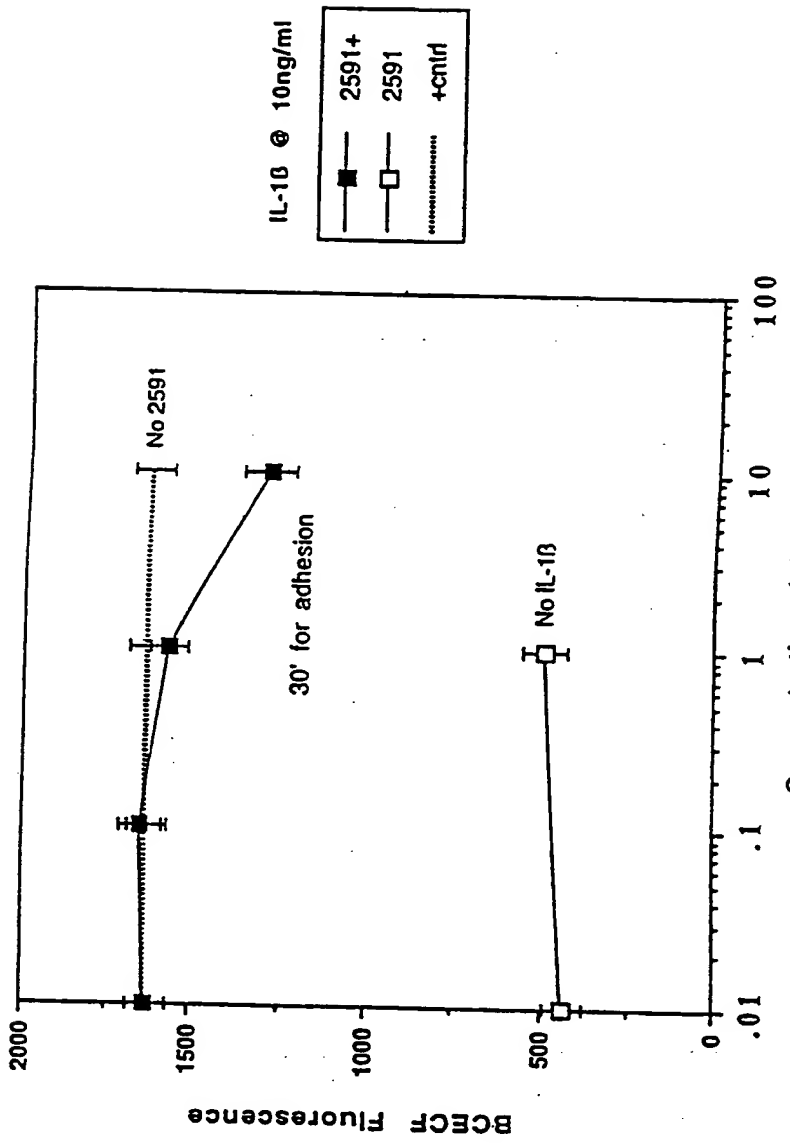


FIGURE 12

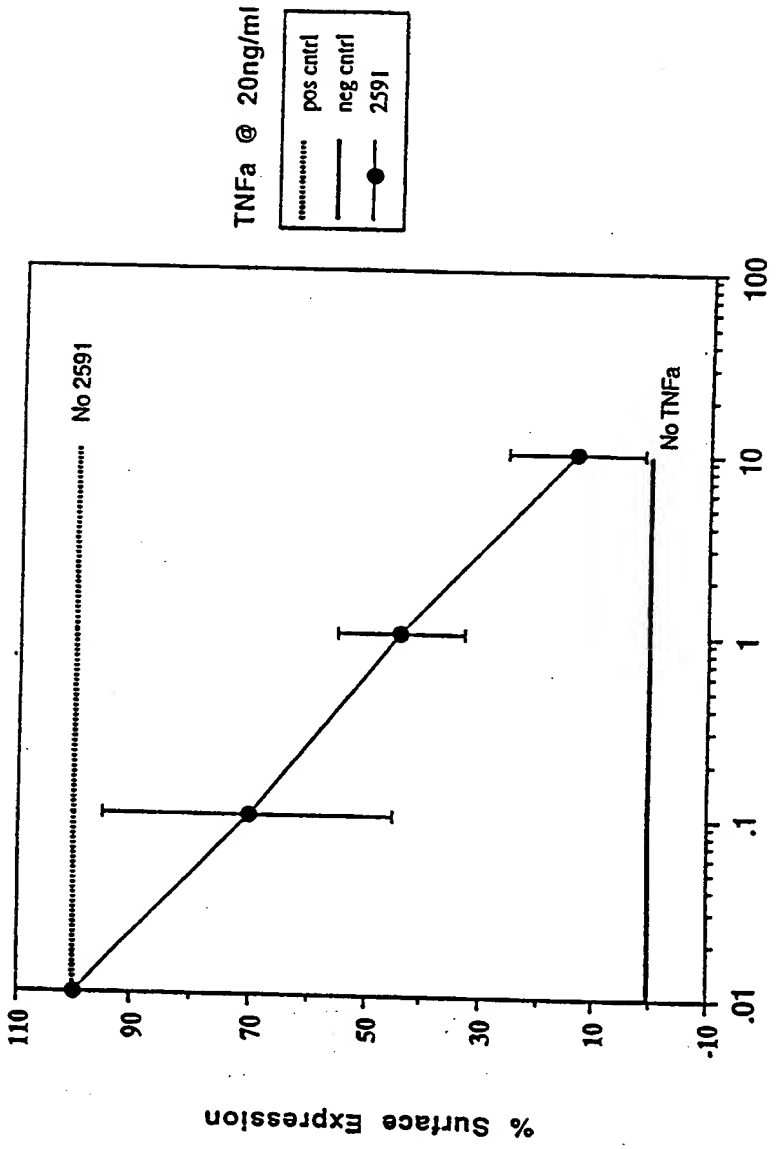


FIGURE 13

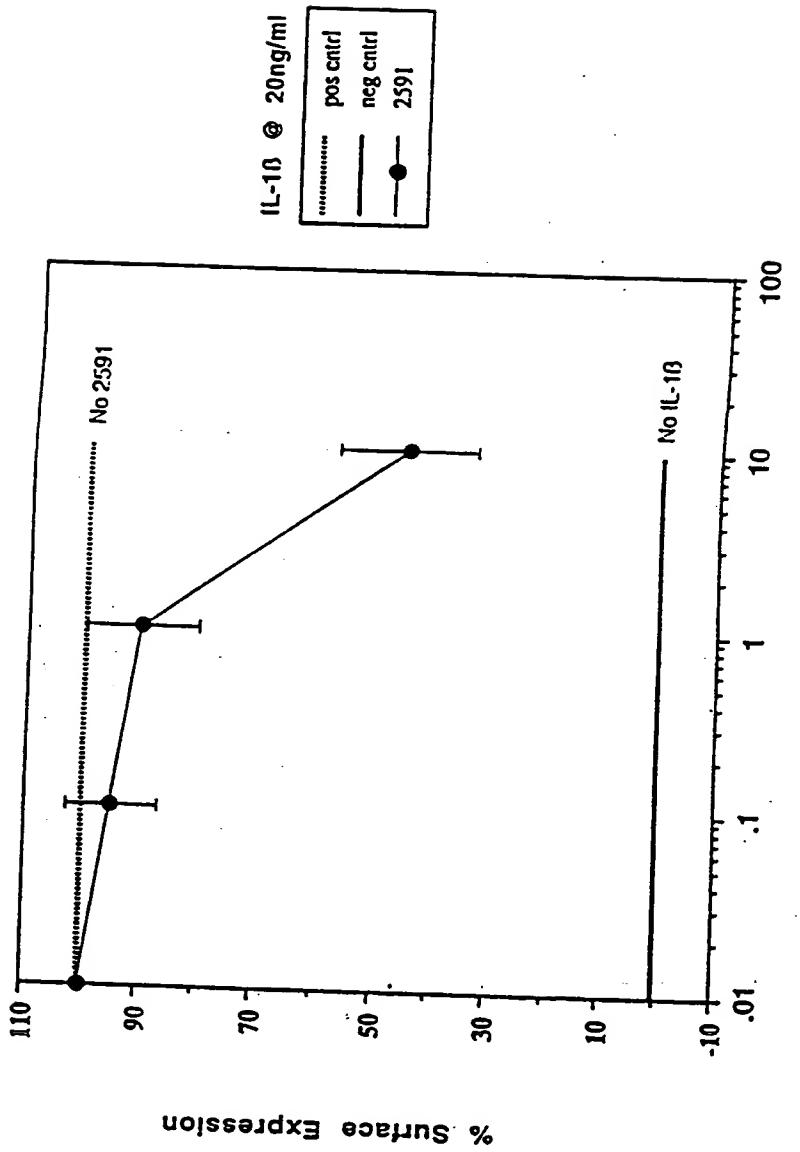


FIGURE 14

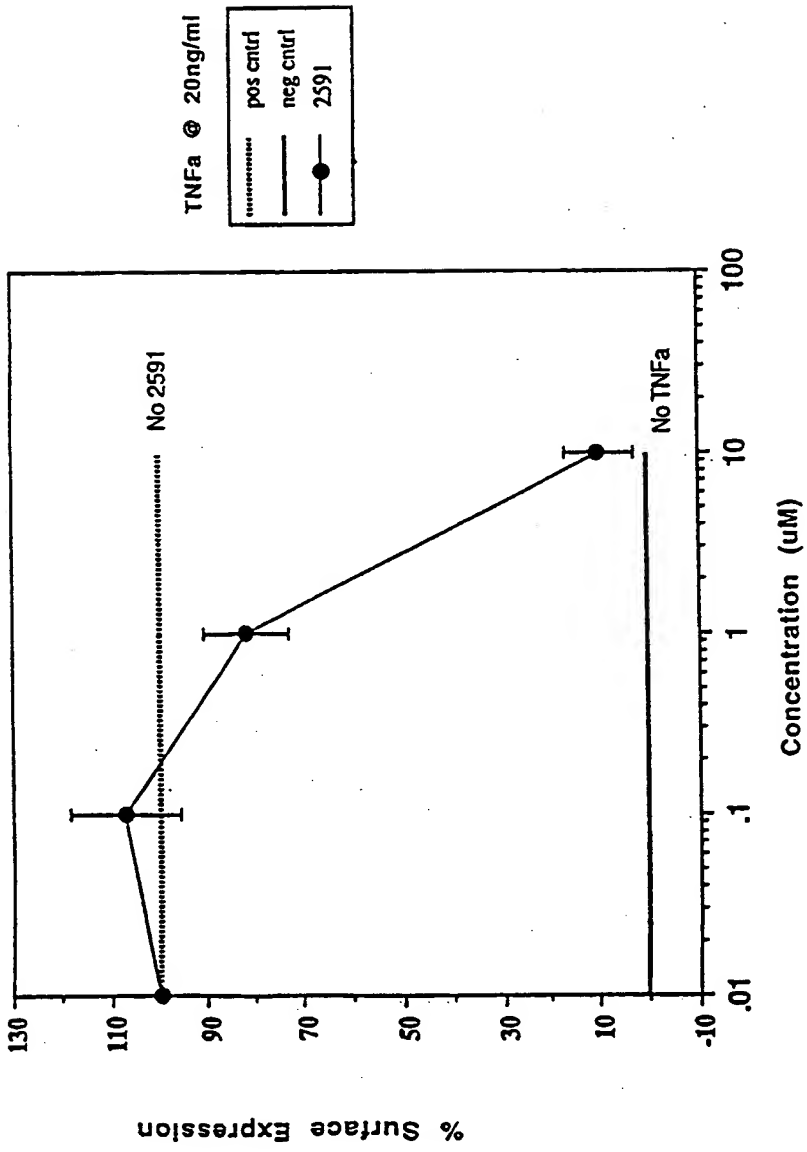


FIGURE 15

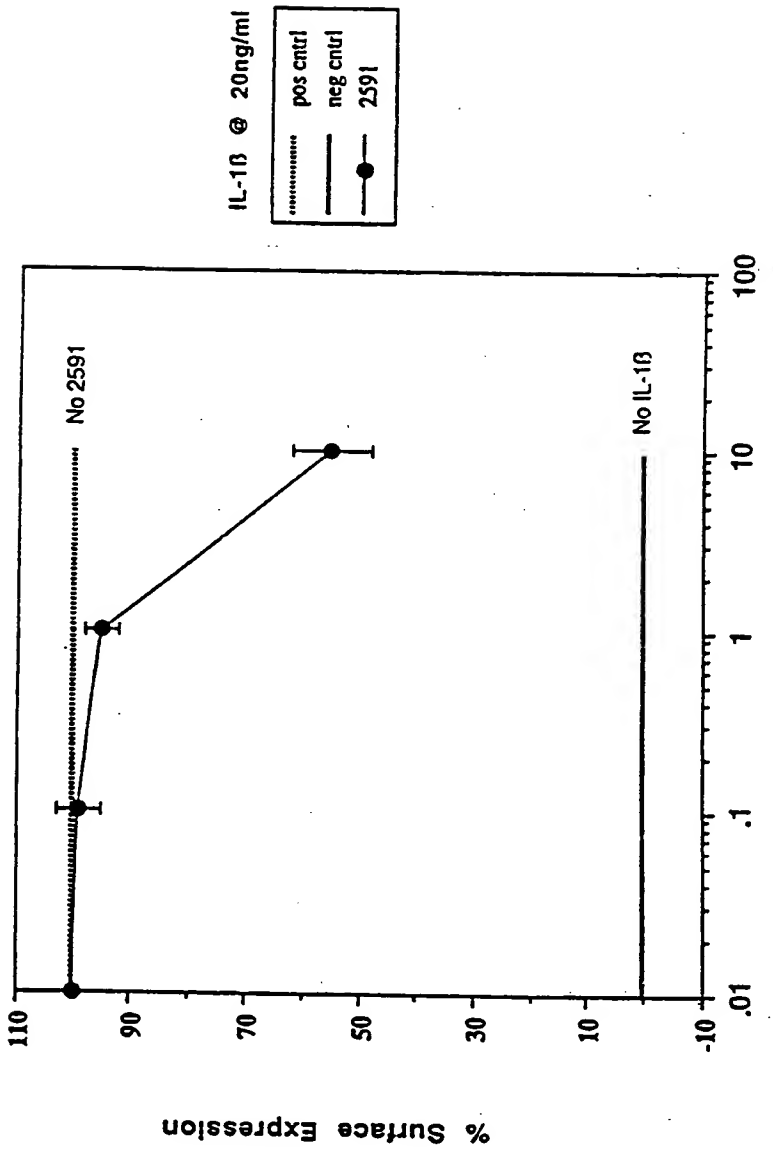

IL-1β @ 20ng/ml
pos cntrl
neg cntrl
2591
No 2591
No IL-1β
% Surface Expression
110
90
70
50
30
10
-10
.01
.1
1
10
100
Concentration (uM)

FIGURE 16

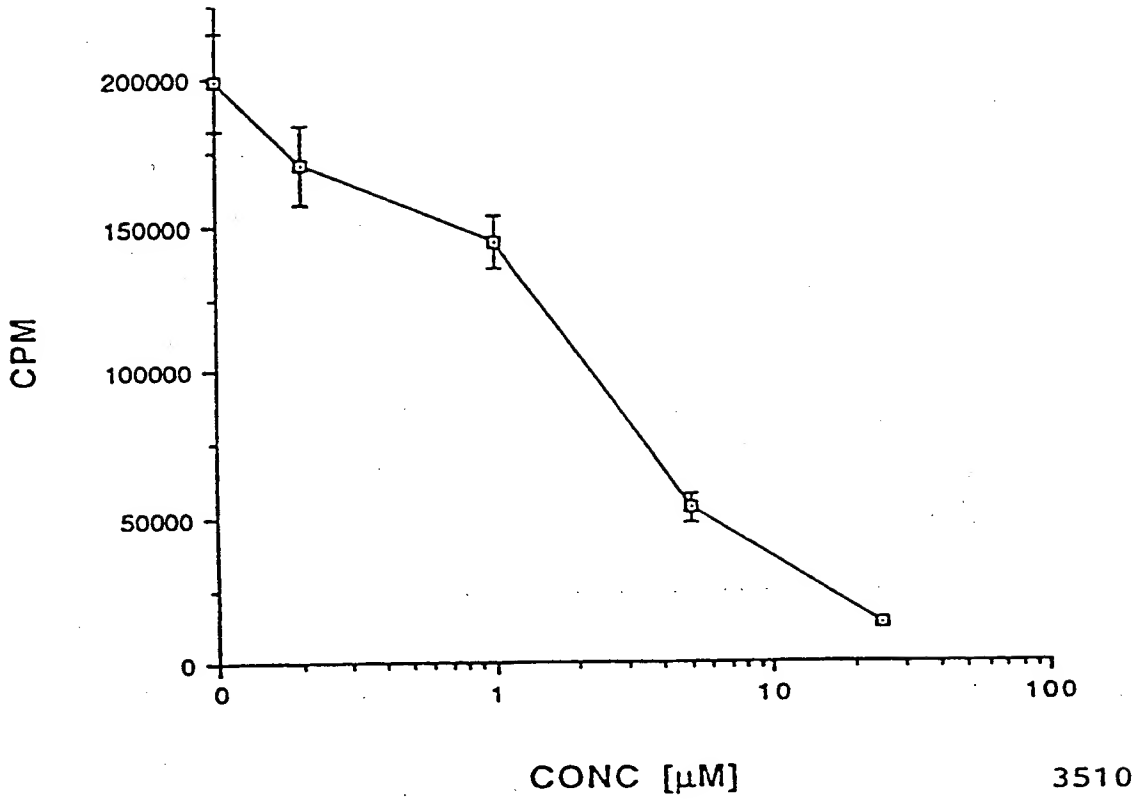


FIGURE 17

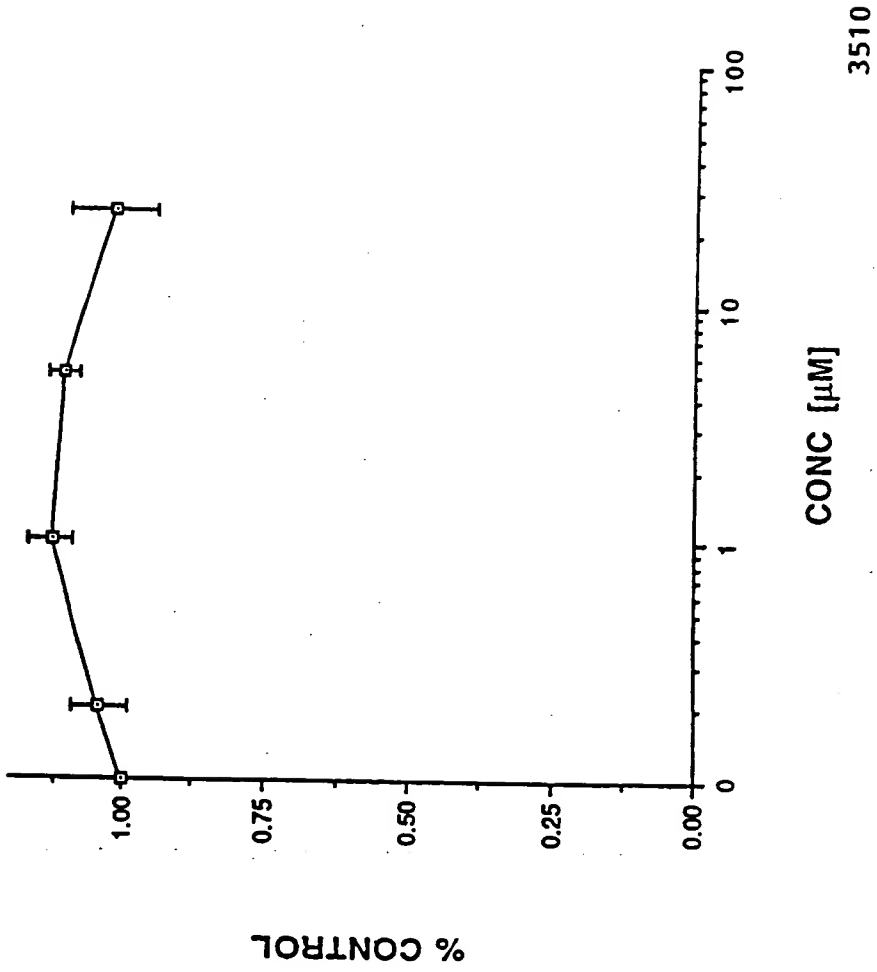


FIGURE 18

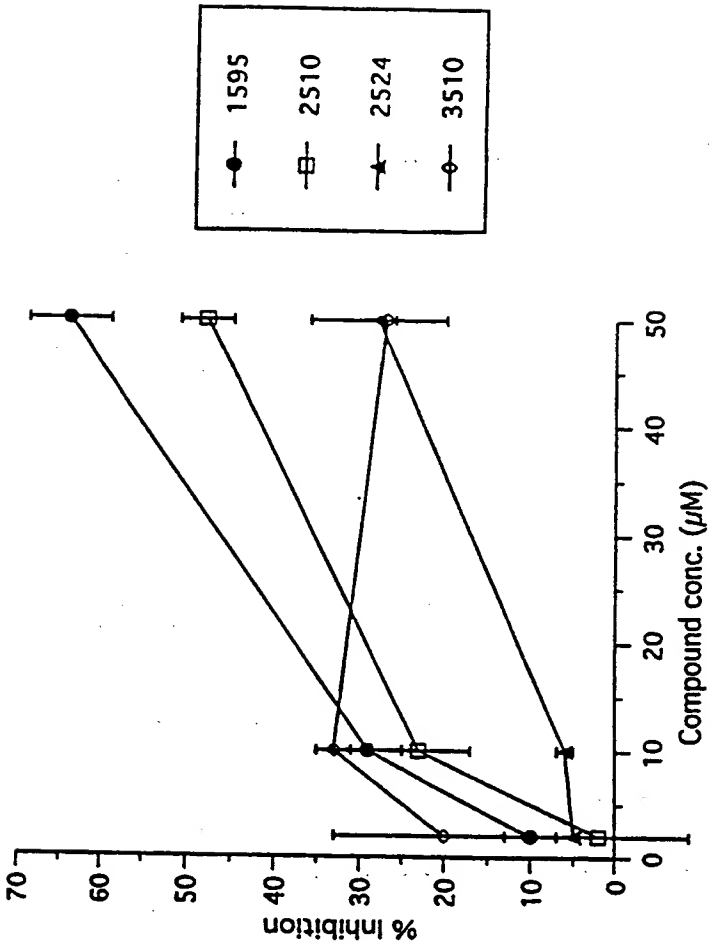


FIGURE 19

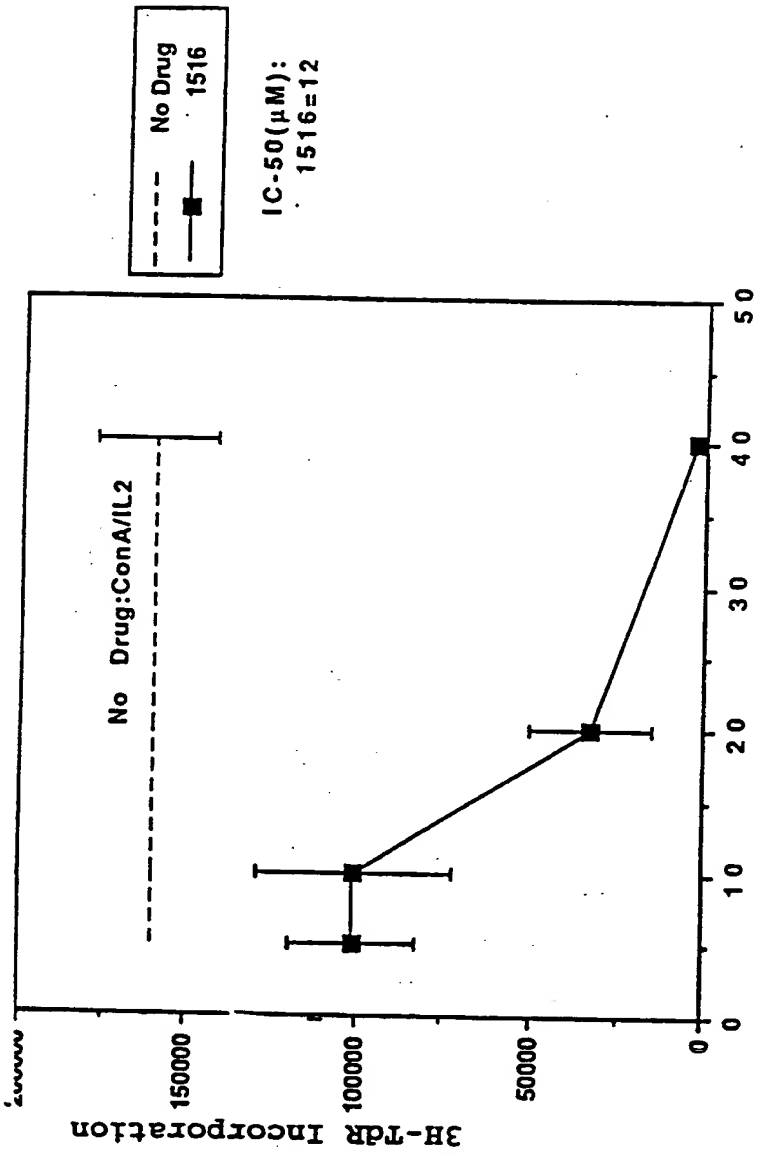


FIGURE 20A

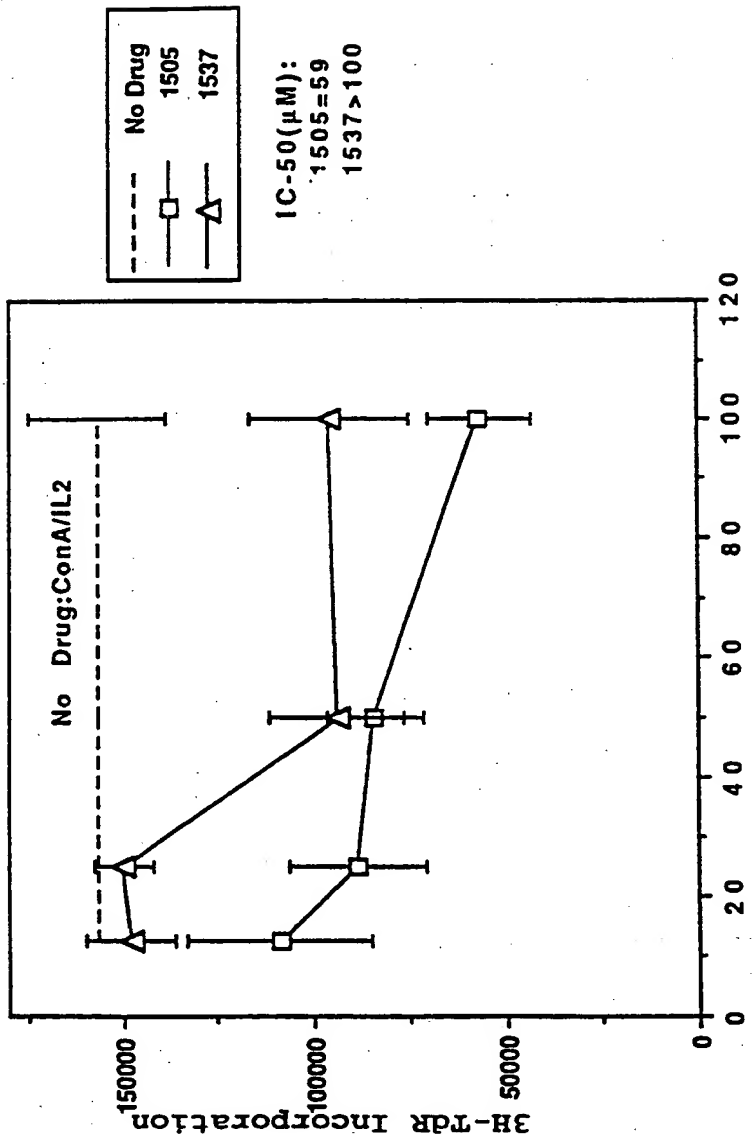


FIGURE 20B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01037

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :544/271, 285, 309; 546/219; 514/263
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/271, 285, 309; 546/219; 514/263

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Zeitschrift fur Chemie, Volume 30, Issued 1990, Lilia Peshakova et al., "Synthesis of some mono- and dioxothymine Uracils and their derivatives" pages 438-439. See compounds 2d, 2a, 3a, 3b.	1-13
Y	Pharmaceutical Research, Issued November 1984, James M. Chapman et al., "Hypolipidemic Agents of Phthalimide Derivatives 6. Effects of Aromatic vs. Non Aromatic Imides", pages 267-269. See Compound 21.	1-13
Y	J. Medicinal Chemistry, Volume 29, Issued May 1986, Utpal Sanyal et al., "New alpha-methylene-gamma lactone derivatives of substituted Nucleic Acid bases as potential Anticancer Agents", pages 595-599, see compounds 4 and 7.	1-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
17 MAY 1995

Date of mailing of the international search report
16 JUN 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
MARK L. BERCH aco

Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01037

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Photochemistry and Photobiology, Volume 37. Issued 1983, Y. J. Lee et al., "Singlet and Triplet Energy Transfer in alpha-diketone derivatives of Uracil and Thymine," pages 381-389, see IX.	1-13
Y	Tetrahedron, Volume 34, Issued 1978, Y. J. Lee, "Fluorescent and phosphorescent pyrimidine labels" pages 2861-2868. See entire document.	1-13
Y	US, A, 4,289,776 (MOHLER et al.) 15 September 1981. See examples 17 and 19.	1-13
A	JP, A, 56-068,681 (NISSEI) 10 November 1979.	1-13, 19
X	NL,A, 73-09709 (CHEMISCHE WERKE ALBERT) 12 July 1972. See Page 2, lines 19, 24, 25.	1-13
A	JP, A, 56-053,680 (HAMARI) 05 October 1979.	1-13, 19
A	JP, A, 58-150594 (DAITO) 03 MARCH 1982.	1-13, 19
Y	US, A, 3,258,491 (LACEY et al.) 28 June 1966. See entire document.	16-18
Y	US, A, 4,203,927 (STAPP) 20 May 1980. See entire document.	16-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01037

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark n Protest

☐ The additi nal search fees were accompanied by the applicant's protest.

☐ N protest accompanied the payment of additional search fees.

F rm PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01037

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07D 473/04, 239/54, 211/88, 239/96; A61K 31/52, 31/505, 31/445

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

Claims 1-12 and 14-19 are not fully searched because they are so broad that their full scope can not be meaningfully searched. Only claim 23 could be meaningfully searched. As a result, claims 1-12 and 14-19 are examined only to the extent that they embrace the species of claim 13.

The compounds of claim 1 have a "core moiety" to which is attached 1-3 R groups. The core moiety is "cyclic or non-cyclic", which covers everything. Even most dependent claims such as claim 10 define one of those choices, but do not require it. Thus, claim 11 defines a vast panapoly of cyclic cores, too broad to be searched, but even claim 11 does not require that the core be cyclic. Claim 12 does narrow the core to three choices. However, in this and all other claims, R is too broad to be effective searched, for essentially the same reason. Thus, even claims such as claim 12 do not require that R be "cyclic or heterocyclic", since claim 12 permits R to still be a group of formula I.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I
Claims 1-19, compounds with xanthine core, plus synthesis, plus use for acute inflammatory disease. Classified in 544/271; 514/263.

Group II
Claims 1-15, compounds with quinazoline core, 544/285.

Group III
Claims 1-15, compounds with glutarimide core, 546/219.

Group IV
Claims 1-29, compounds with uracil core, 544/309.

Group V
Use for AIDS or ARC, 514/various.

Group VI
Use for alcoholic hepatitis, 514/various.

Group VII
Use for allergies due to degranulation of mast cells and basophils, 514/various.

Group VIII
Use for angiogenesis, 514/various.

Group IX
Use for asthma, 514/various.

Group X
Use for atherosclerosis, 514/various.

Group XI
Use for autoimmune thyroiditis, 514/various.

Group XII
Use for coronary artery disease, 514/various.

Group XIII

Use for glomeruls nephritis, 514/various.

Group XIV
Use for hair loss or baldness, 514/various.

Group XV
Use for HIV-associated dementia, 514/various.

Group XVI
Use for IBD, 514/various.

Group XVII
Use for insulin dependent diabetes mellitus, 514/various.

Group XVIII
Use for lupus, 514/various.

Group XIX
Use for malignancies, 514/various.

Group XX
Use for MS, 514/various.

Group XXI
Use for myelogenous leukemia, 514/various.

Group XXII
Use for organ response to cytotoxic therapy, 514/various.

Group XXIII
Use for hematopoietic response to cytotoxic therapy, 514/various.

Group XXIV
Use for osteoarthritis, 514/various.

Group XXV
Use for osteoporosis, 514/various.

Group XXVI
Use for peridontal disease, 514/various.

Group XXVII
Use for premature labor secondary to uterine infection, 514/various.

Group XXVIII
Use for psoriasis, 514/various.

Group XXIX
Use for restenosis, 514/various.

Group XXX
Use for rheumatoid arthritis, 514/various.

Group XXXI
Use for sleep disorders, 514/various.

Group XXXII
Use f r septic shock or sepsis syndrome, 514/various.

Group XXXIII
Use for scleroderma, 514/vari us.

Group XXXIV
Use for stroke, 514/various.

Group XXXV
Use for transplant rejection, 514/various.

Group XXXIV
Use for chronic Inflammatory Disease, 514/various.

The compound groups I-II-III-IV are clearly structurally distinct as seen by their markedly different cores. Group I is distinctive in having 2 heterocyclic rings; Group III is distinctive in having a 6 membered ring with only one heteroatom; Group II is distinctive in having a fused benzoring. The differing utilities are distinct because they involve different diseases, different types of origins, and different parts of the body. For example, some arise as a result of bacteria (sepsis) some from a virus (AIDS) and some not from an infectious agent (coronary artery disease). They involve different parts of the body, e.g. brain (stroke), liver (alcoholic hepatitis), intestines (IBD), skin (psoriasis), and others. Some are cancers (malignancies) and some are considered extremely difficult or impossible to treat (HIV dementia, sepsis, CF), and others are more amenable (asthma).

Applicants should thus note that the search is done in the claim 13 species, their preparation, and use for acute inflammatory disease.